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EXPERIMENTAL STUDIES ON THE PATHOGENESIS OF IDIOPATHIC UVEITIS
REPORT 2. INOCULATION-TEST WITH HUMAN DONORS OF PATIENTS OF IDIOPATHIC UVEITIS

Eiichi YOSHIDA

Micro-biological Department, Faculty of Medicine, Kyoto University,
Ophthalmological Department, Faculty of Medicine, Kyoto University

The pathogenesis of idiopathic uveitis has been studied by many investigators, and various etiologies have been proposed, but none of them has been definitely proved. Since idiopathic uveitis is suspected of having some possible relationship with a virus, precise ophthalmological studies are in order. Many studies have so far been done on the clinical and histological findings of uveitis provoked in rabbit eyes injected with materials from patients. In 1930 Takahashi and Tagami presumed that the causative agent was probably a virus. In recent years, notable advances have been made in virus research. Ikui and Remky suggested that idiopathic uveitis might be due to viral infection, though no causative agent had been isolated. However, there have been noly a few viral and serological studies. In fact there is found in the literature only that of Sugiura (1953), who isolated a strain by serial passage in brains of mice.

In previous papers, the present author reported the following results. 1) Inoculation of normal allantoic fluid from 11～12 day-old chick embryos (Alf.-11～12 days) at pH. above 7.0, did not cause inflammation in rabbit eyes. 2) Inoculation of Alf.-older than 13 days at pH. below 7.0, caused uveitis always in 8～32 days. 3) Uveitis developed following inoculation of the vitreous humor of an inflamed eye into the vitreous of a second rabbit. 4) The experimental uveitis of rabbits was probably due to the antigen-antibody reaction—perhaps allergy—between antigen which had remained in the uveal tissue, and systemic and local antibodies. The present report describes the successful results of viral and serological research. The author used materials from four patients (Harada type) in these experiments.

Materials and Methods

Animals. Colored rabbits, weighing approximately 4 lb., developing chick embryos (egg) of 8～11 days and mice of body weight 6～8 g., supplied by commercial breeders.

Materials. Subretinal fluid (S.R.), aqueous humor (A.Q.) and spinal fluid (L.) of the patients.

Method used were chiefly passage through rabbit eyes (intravitreously), cultivation into the allantoic cavity of eggs, blind passage into mouse brains, and immunological investigations. The amount injected into rabbit eyes and egge was 0.2 ml. of various dilutions of the materials, and in mice 0.025 ml..

Preliminary experiment. A 10-fold saline dilution of S.R. from a case of idiopathic

retinal detachment was inoculated into the right eyes of 2 rabbits, and simultaneously the same material, containining penicillin 200 unit/ml. and streptomycin 1 mg./ml., into the left eyes. The right eyes showed no changes during the 30 days after inoculation, while the left eyes showed widespread atrophy and depigmentation of the retino-choroid. From these findings, it might be considered that penicillin and streptomycin, suspended in the S.R. of a case of idiopathic retinal detachment, have some toxic action on the uvea of rabbits.

Experiment 1

Case. 31-year-old womon (Harada type). S.R. was aspirated 5 months after onset of the disease.

The results are briefly as follows.

1) Intravitreal inoculation of the S.R. of the patient into rabbit eyes caused uveitis and neuritis for 3 passages. The incubation period was 5～18 days.

2) The vitreous of the inflamed eyes of 2 rabbits of the 1st and 2nd generation was diluted with saline to give a 10-fold suspension. Each suspension was inoculated into the allantoic cavity of an egg, in which after 3 and 4 passages respectively the Alf.-13～15 days were centrifuged for 20 minutes at 3000 r. p. m.. Each supernatant, mixed with penicillin and streptomycin, was immediately inoculated group by group into 2 rabbits, which developed uveitis 6～29 days after inoculation.

3) The same suspension was inoculated into eggs. After 3 passages the cultivated Alf.-15 days was prepared in the similar procedure to a supernatant, which was inoculated not only into 2 rabbits, but also into eggs. In the latter case, after 3 passages again the Alf.-14 was inoculated into 2 rabbits, which developed uveitis after 31 days.

4) Blind passage through mouse brains of S.R. and the vitreous of inflamed rabbit eyes was unsuccessful.

5) The cultivated Alf.-14 days was inoculated into 3 rabbits, which had entirely or almost recovered from uveitis. None of the 3 eyes developed any inflammation, and therefore the immune-test was found to be successful.

6) The vitreous of inflamed rabbit eyes failed to agglutinate hen red cells.

7) Untouched eyes showed no changes.

8) Following serial passage through eggs, no macroscopic abnormalities were seen.

Experiment 2

Case: 61-year-old man (Harada type). L. and A.Q. were aspirated 33 days after onset of the disease.

The results are briefly as follows.

1) L. was inoculated into 4 rabbits, which showed no changes during 11～117 days after inoculation.

2) L., mixed with penicillin and streptomycin, was inoculated on the chorioallantoic membrane of eggs for 3 passages without any abnormalities.

3) The Alf-12 days after 2 passages, failed to agglutinate goat red cells.

4) A 2-fold A.Q.-dilution was inoculated into 2 rabbits, which developed no clinical signs.

Experiment 3

Case. 41-year-old woman (Harada type). S.R. was aspirated 36 days and L. 37 days after onset of the disease. The results are briefly as follows.

A) Passage of S.R. through rabbit eyes.

1) A 10-fold S.R.-dilution was inoculated intravitreously into 3 rabbits, which developed severe uveitis after 6~7 days.

2) Then a 10-fold dilution of the vitreous of an inflamed rabbit eye was inoculated into 2 rabbits. The results were unsuccessful.

3) The vitreous of another inflamed rabbit was diluted to 10 and 1000-fold suspensions with saline. These vitreous suspensions were used to form 50% solutions of patient serum and of control human serum, and the presence of neutralizing antibodies in the patient's serum was inhvestigated by means of intravitreal inoculation into 10 rabbits. The 4 rabbits which received vitreous suspension only developed no uveitis, and the 4 rabbits which received 50% patient serum in vitreous suspension and the 2 rabbits which received 50% control human serum in vitreous suspension developed severe uveitis, complicated with cataract. Consequently the neutralization-test in this method was insignificant.

4) Six inflamed rabbit eyes, without cataract, did not develope atrophy or depigmentation of the retino-choroid.

5) Untouched eyes had no clinical sign.

B) Passage of L. through rabbit eyes.

Intravitreal inoculation of L. into 3 rabbits caused no uveitis after 31, 31, and 63 days.

C) Cultivation of S.R. in the allantoic cavity of eggs.

1) The Alf-17 days after 7 passages was inoculated into 2 rabbits, which revealed severe uveitis after 5 and 10 days.

2) Cutaneous reaction of inflamed rabbits.

The Alf.-14 days after 11 passage was cutaneously injected in 6 inflamed rabbits. In 2 of them the reaction was positive.

3) The Alf.-16 days after 13 passages and its filtrate (by Chamberland L-3) were each inoculated into 2 rabbits. One of each group develped uveitis.

4) The Alf.-16 days after 13 passages was failed to agglutinate goat and rabbit red cells.

5) The Alf.-12 days after 18 passages was inoculated into 2 rabbits. One of them developed uveitis after 22 days.

6) The same specimen was observed by electron microscope, but viruslike particles were not found.

7) None of the eggs used in serial passages showed any abnormalities macroscopically.

8) Untouched eyes showed no changes.

D) Inoculation of L. into the allantoic cavity.

The Alf.-17 days after 7 passages was inoculated into 2 rabbits, which developed severe uveitis in 5 days. One of them later developed retinochoroidal atrophy.

Experiment 4

Case. 44-year-old man (Harada type). S.R. of both eyes was aspirated 39 days after onset of the disease.

The results obtained are as follows, and the details of the data are presented in tables 1 and 2.

Table 1. subretinal fluid of the right eye (3x)

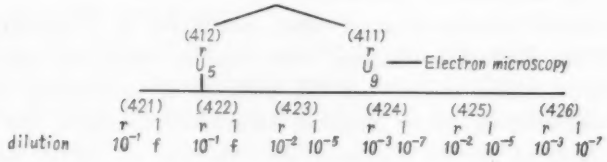
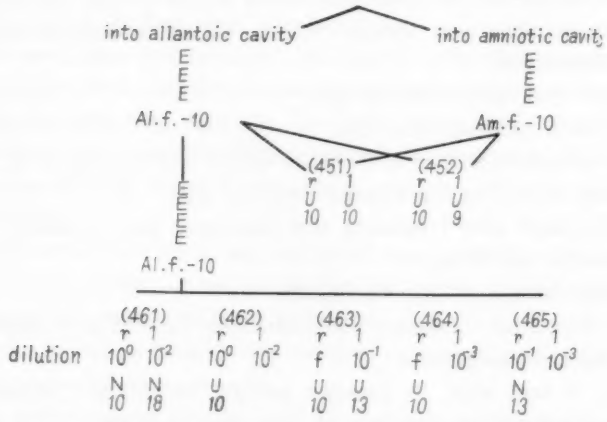


Table 2. subretinal fluid of the left eye (8x)


into allantoic cavity
into amniotic cavity
E E E
Al.f.-10
Am.f.-10
(451) (452)
r l r l
U U U U
10 10 10 9
Al.f.-10
(461) (462) (463) (464) (465)
r l r l r l r l r l
dilution
10⁰ 10⁻² 10⁰ 10⁻² f 10⁻¹ f 10⁻³ 10⁻¹ 10⁻³
N N U U U U N
10 18 10 10 13 10 13

( ) : *rabbit number*, r: *right eye*, l: *left eye*, x: *dilution*, Alf.: *allantoic fluid*, Amf.: *amniotic fluid*, U.: *uveitis*, N.: *neuritis*, number of U. or N..: *incubation period*, E.: *chick embryo*

A) Passage through rabbit eyes of S.R. from the right eye

A 3-fold S.R. dilution of the right eye was inoculated into 2 rabbits, (411) and (412), which developed uveitis after 9 and 5 days respectively. The vitreous of rabbit (412) was not only diluted with saline to 10^{-1}~10^{-7}-fold suspensions, but also filtered through Chamberland L-3 filter. Both these suspensions and filtrate were inoculated into both eyes of 6 rabbits, as showed in table 1. No uveitis developed in any of the 12 eyes.

B) Inoculation of S.R. from the left eye into the allantoic and amniotic cavities.

An 8-fold dilution of S.R. was inoculated not only into the allantoic cavity, but also

into the amniotic cavity.

1) After 3 serial passages in both series, the Alf.-10 days and the amniotic fluid-10 days were inoculated into 4 eyes of 2 rabbits, the former into the right eyes, the latter into the left eyes. All 4 eyes developed severe uveitis in 9～10 days, while 4 control eyes showed no changes.

2) After 7 passages, the Alf.-10 days was diluted with saline to give suspensions in concentrations of 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}. or filtered through Chamberland L-3 filter. Each of these suspensions and filtrate, always in 0.2 ml. quantities, was inoculated into both eyes of 5 rabbits. In 7 eyes of 4 rabbits uveitis and neuritis developed 10～18 days after inoculation.

3) None of the eggs in either series showed any abnormalities.

C) Immunological investigation

Complement fixation test and precipitation test, between the cultivated Alf. after serial passages and patient sera 55～76 days after onset, were negative. The Alf. after 7 passages did not agglutinate hen red cells.

D) Electron microscopic observation

The electron micrograph of the optic nerve of rabbit (411) showed vacuolization of the myelin sheath of the nerve fiber, but paticles of viruses were not found.

Conclusion

Intravitreal inoculation into rabbit eyes of the S.R. from patients with idiopathic uveitis induced uveitis and neuritis which might very possibly be due to heterologous protein as the causative agent. But it is difficult to cause uveitis in subsequent intravitreous passages of the vitreous humor from an inflamed eye. Since rabbit uveitis is probably due to some causative agent, very probably virus, the difficulty in serial passage may be due either to deficiency or variability in the tropism of the inoculated viruses, or in their scarcity. Therefore this experimental method is not to be desired.

As reported in previous papers, inoculation with normal Alf.-11～12 days causes no inflammation in rabbit eye, but inoculation with normal Alf.-older than 13 days at pH. below 7.0 always causes uveitis in 8～32 days. It is considered that the pathogenicity of cultivated Alf.-10～12 days may be demonstrated by developement of uveitis in inoculated rabbit eyes. Consequently in experiment 4, some virus is surmised to be isolated in the cultivated Alf., but not identified by complement fixation, precipitation, or hemagglutination tests. Positivity of the immune test in experiment 1 and of the cutaneous reaction in experiment 3, may show isolation of viruses. All other immunological investigations were negative. A future report will describe the histological findings of rabbit uveitis.

(Published in Acta Ophth. Jap. 61:1211, 1957 in Japanese.)

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THE BIOMICROSCOPY OF THE HUMAN EYE USING POLARIZED LIGHT: FINDINGS IN NORMAL CORNEA

Saiichi MISHIMA

Department of Ophthalmology, Tokyo Medical and Dental University

Introduction

By means of the polarized light technique, the fine structure of the ocular tissues has so far been successfully elucidated. On the isolated cornea, some remarkable studies were made recently from the standpoint of crystal optics by Stanworth & Naylor[3], Naylor[6] and Kikkawa[2)3)4)]. As for the living human cornea also, Nordenson[7] estimated the magnitude of birefringence by checking interference color. Cogan[1] also described the dark cross on it, and reviewed relevant literatures on the dark cross of the isolated cornea.

The biomicroscopy using polarized light was first made by Koeppe[5], who described an interwoven network of lines in the cornea, assuming these lines to be a cement substance connecting corneal lamellae.

In spite of his elaborate work, the polarized light technique in the biomicroscopy of the human eye has not been fully established hitherto. The incompleteness of this technique might disturb the extensive application for the clinical purpose, which would be highly appreciated by ophthalmologists.

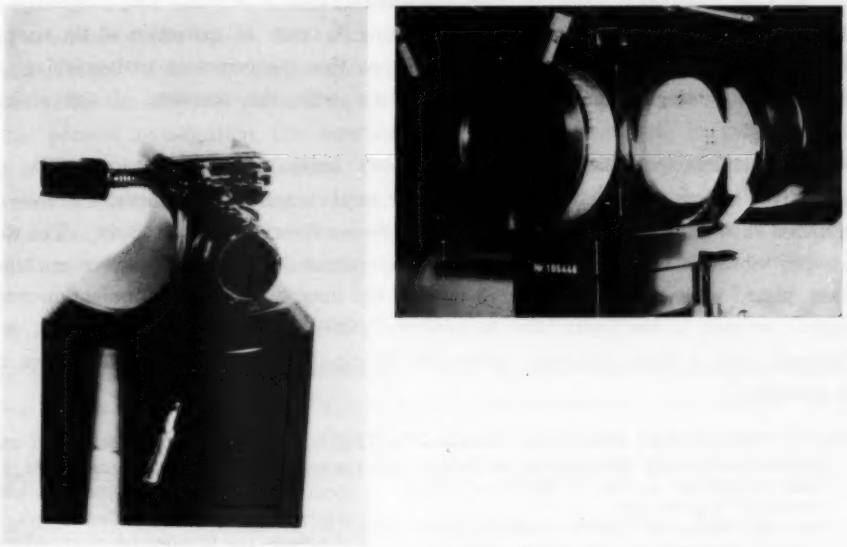
In the present investigation, attempt was first made to establish a technique of the biomicroscopy using polarized light for the clinical application, and then by the use of this technique, the structure of the living human cornea was studied. The present report will deal with the actual technique of this method and some phenomena newly found with it concerning the so-called dark cross, the interference color and also the fibrous structures.

Equipment

The equipment consists of the Littmann's slit lamp biomicroscope and two polarizing filters (Dichrom for microscopic use, Mitsubishi Elect. Co.). One of the filters, the polarizer, is placed in front of the illuminating prism, and the other serves as the analyser attached to the front of the objective lens of the binocular microscope. The polarizer

can be rotated through 180° and the analyser through 360°. The direction of vibration of light passing through each filter can be known by the rotating angle of the filter. The attachments containing those filters are illustrated in Fig. 1 a and 1 b.

Fig. 1: a) Polarizer, b) Analyser



Method of Observation

The illumination-observation angle and the crossing of the polarizer and the analyser. By analogy with the conventional technique of the polarizing microscope, the observation with crossed polars (the polarizer and the analyser) is required here. In observing reflected polarized light, however, some considerations are needed as to the crossing of the direction of the analyser against that of reflected light. For the purpose of the simplicity, the observation of reflected polarized light from the surface of transparent isotropic substance, e. g. a strain-free glass, is considered as a model-case in the following description.

According to the Fresnel's law of reflection, a plane-polarized light is reflected from the surface of the specimen with a phase change of 180° or 0°, if the total reflection is ignored. The reflected light is therefore plane-polarized, its direction of vibration being rotated in general, depending on the magnitude of incident angle, the refractive index of the transparent specimen and also the direction of vibration of incident light. Even if the refractive index and the vibration direction of incident light are assumed to be definite, the direction of analyser still remains unsettled, unless the magnitude of incident angle is determined. Therefore, the control of the magnitude of incident angle, which corresponds to the illumination-observation angle of the present apparatus, is of prime importance in this technique.

In the binocular microscopy some particular problems may arise due to the nonparallelism of the optic axes of two microscopes. When two beams, reflected at differently inclined surfaces, fall into each microscope, the directions of vibration of crossed analysers inevitably differ from each other. The difference between the azimuths of the analysers of two optic systems were plotted in Fig. 2 against the magnitude of the illumination-observation angle of the apparatus in case of reflection at the surface of a glass plate. It is clearly indicated in this figure, that the errors in extinguishing reflected rays, with a single analyser, become larger with the increase of the obliquity of illumination.

The incident polarized ray is reflected at many successive layers with different refractive indices in different ratios of vectoral amplitudes. Therefore, the reflected polarized rays from those layers vibrate in different directions respectively. The difference between vibration directions of polarized rays, reflected at two successive surfaces of a glass plate, were plotted in Fig. 3 against the magnitude of the illumination-observation angle. As seen in the figure, the satisfactory extinction of the reflected rays is hardly obtained, with a single analyser, unless the illumination-observation angle is kept as small as possible.

Fig. 2. The difference between the vibration directions of crossed analysers in the binocular microscope, in case of reflection at the surface of a glass plate;
Ordinate: difference between directions of crossed analysers of two optic systems,
Abscissa: the illumination-observation angle of the apparatus
The direction of vibration of incident light is set at 30° to the plane of incidence by means of a specially deviced attachment. The angle of rotation of the analyser was measured with a vernier with 1° scale.

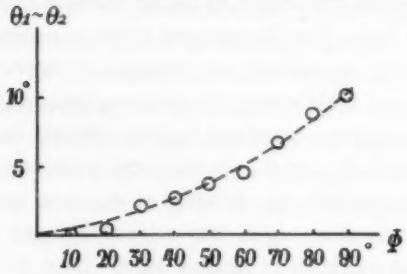
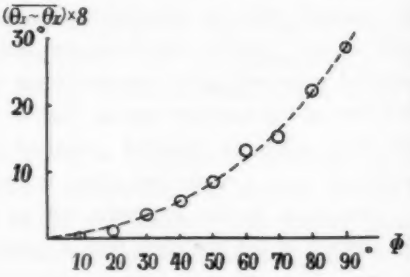


Fig. 3. Reflection of polarized light at two successive surfaces of a glass plate;
Ordinate: $(\theta_I \sim \theta_{II})$ the difference between vibration directions of reflected rays,
Abscissa: the illumination-observation angle of the apparatus
The vibration direction of incident light is set at 30° to the plane of incidence.



Judging from the facts above mentioned, the standard technique should consist in setting the illumination- observation angle at 0° and in crossing the polars. In the present apparatus, when this angle is set at 0°, the actual angle between the illumination- and observation-axis is 5°; this value can be ignored. If it is inconvenient for the observation to set this angle at 0°, an oblique illumination may be used. In this case, however, the

illumination-observation angle should be kept as small as possible. So far as the accuracy for the clinical use is concerned, it may be varied within 30° without serious blur of the observed figures. The same procedure must be performed in case of the observation of reflected rays from the surface of the eye. On that occasion, the curvature of the corneal surface and the convergency of beams of slit lamp might cause the errors.

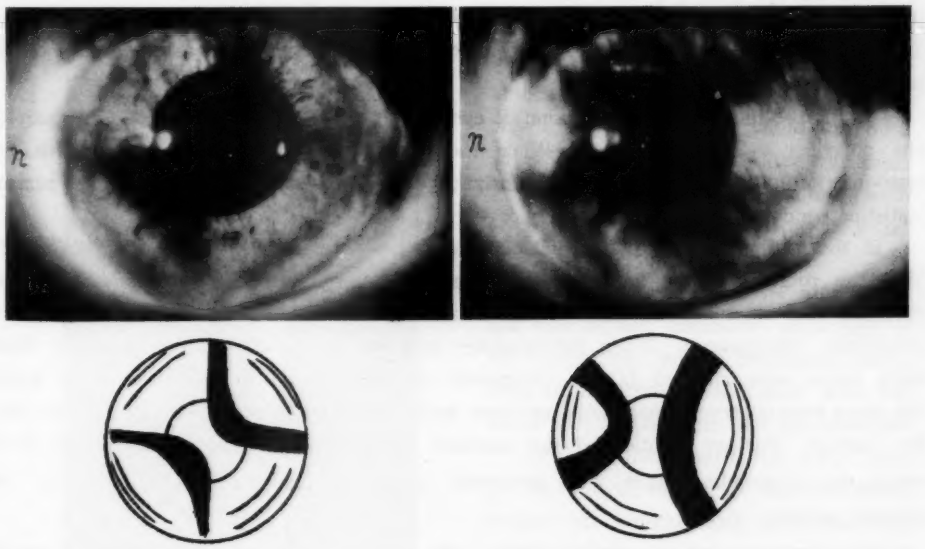
The direction of observation. The direction of observation as well as the observation-illumination angle is also of particular importance, because the optic effect of anisotropic substance varies considerably, depending on the direction of the propagation of rays within it. In the present investigation, the direction of observation was set, in general, to be normal to the surface of the corneal tissues. The dark hyperbola and the interference color were observed from the direction normal to the vertex of the cornea.

The azimuth of the polarizer. The azimuth of the polarizer was expressed in a counter-clockwise angle in reference to the horizontal, as seen from the side of the observer.

So-called Dark Cross of Cornea (Dark Hyperbola)

The observation with diffuse illumination. The so-called dark cross-shaped figures of the living cornea are illustrated in Fig. 4 a and 4 b for the azimuth of 0° and 45° of the polarizer respectively. As seen in the figure, the so-called dark cross is not a complete cross, but is composed of a set of hyperbolic figures separated in the central region. It

Fig. 4. The dark hyperbola observed with diffuse illumination, and their schematic illustrations; n indicates the nasal side. The azimuth of the polarizer; a) 0° or 90°, and b) 45° or 135°.



may, therefore, be adequate to express this figure as "the dark hyperbola" instead of "the cross".

With the azimuth of 0° or 90° of the polarizer, the hyperbolic figures were observed in the naso-inferior and temporo-superior quadrants of the cornea, their arms being directed at approximately parallel to the direction of the polarizer and the analyser respectively. The arms of the hyperbolic figures were about 1 mm. wide in the paracentral region, and they became narrower toward the periphery with average width of about 0.6 mm.. The figures were rotated with the rotation of the polarizer and analyser in the same direction and to an equal extent. When the polarizer was set at 45° or 135°, they appeared in majority of cases to be devided into the nasal and temporal halves. In this case the distance of the vertices of the hyperbolic figures was larger, and the arms in the periphery became wider than that observed with the azimuth of 0° or 90°. With the rotation of the polarizer and the analyser, therefore, the brightness of the peripheral cornea was much changed, while the central cornea instead was subjected to no remarkable variation of brightness. The appearrance of the figures in the definite quadrants described above may suggest some asymmetrical structure of the cornea.

The hyperbolic figures, when they were observed in the foreground of the lens, were more clearcut than when seen in front of the iris. This phenomenon may possibly be explained by the fact that the iris, as stated by Cogan, seriously depolarizes the plane-polarized light but the lens surface does not.

The obliquity of the illumination, the direction of the observation and the version of the eyes have also serious influences on the shape and the site of the figures. By increasing the obliquity of the illumination, the figures became much blurred; for instance, with the azimuth of 0° or 90° of the polarizer, they were seriously deformed, when the observation-illumination angle was over 30°. The more serious deformation was observed with the azimuth of 45° or 135°.

As for the influence of the version of eye, an instance seen with 0° or 90° azimuth of the polarizer is presented here: When the eye was turned up- and lateral-wards, the naso-inferior half moved toward the periphery while the temporo-superior half became indistinct, moving centralwards, and vice versa.

If the analyser was rotated independently, the figures were immediately deformed: With the rotation of the analyser, the arms of the figures were rotated in the same direction and remained as poorly defined dark areas, though central part of the figures completely disappeared. When the analyser was set at parallel to the polarizer, these dark areas were situated in the peripheral region diagonal to the position in which the arms were observed previously, and they were separated by several colored bands from the limbus. Further rotation of the analyser caused the rotation of these areas, from which the hyperbolic figures were reformed, when the analyser was set again at the crossed position.

The observation of the optical section. The optical section of the peripheral cornea

revealed a dark gap corresponding to the arms of the hyperbolic figures as shown in Fig. 5 a and 5 b. On either side of the gap, the deep layer became brighter, and adjacent to the limbus, this bright layer appeared to occupy almost the deeper two-thirds of the whole thickness.

Fig. 5. The optical section of the peripheral cornea, the azimuth of the polarizer; a) 0° or 90°, and b) 45° or 135°. The dark gaps correspond to the arms of hyperbolic figures.

Fig. 6. The optical section of the peripheral cornea observed with parallel polars, the azimuth of the polarizer; 0° or 90°.

The dark gap also moved together with the rotation of the polarizer and the analyser. In this case, the change in brightness of the optical section was observed only in the deep layer, while the dark superficial layer showed little variation. The hyperbolic figures, therefore, seem to be intimately related to the structure of the deep layer of the cornea.

The optical section of the central cornea was darker, compared with that of the peripheral, and its brightness underwent little variation associated with the rotation of the polarizer and the analyser.

With parallel polars, the deep layer of the optical section of the peripheral cornea, situated diagonally to the direction of the analyser and the polarizer, appeared

to be dark. This dark layer corresponded to the dark area observed with diffuse illumination, and extended more peripheralwards. (Fig. 6) At the extreme periphery, this dark layer occupied almost a half of the whole thickness of the cornea, its deepest layer being tinted faintly.

Interference Color

The colored bands in multilayers, which appeared to lie on the surface of the iris, were observed in the peripheral region diagonal to the arms of the hyperbolic figures. They were arranged from the periphery in the order of dark red, yellow, blue and dark red fading gradually toward the center. With the use of a monochromatic filter, they were replaced by dark and bright stripes, similar to these stripes distinctly visible in Fig. 4 a and 4 b.

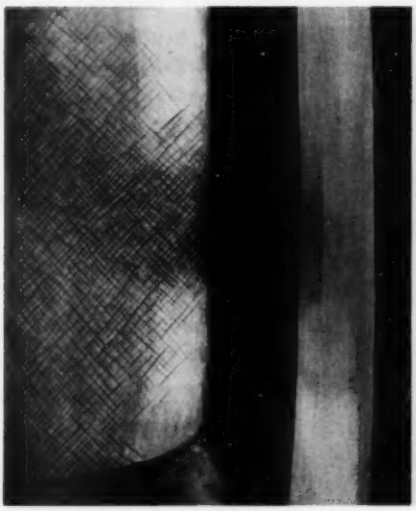
The optical section of the cornea revealed no such remarkable coloration, but careful examination showed that the deepest layer of the peripheral cornea was faintly tinted.

These colored bands as well as the hyperbolic figures were seriously influenced by the directions of both observation and illumination.

With parallel polars, the arrangement of colors was very much altered. In view of the optical theory, the interference colors produced by the use of parallel polars are complementary to those by crossed polars. In the present case, the colored bands appeared to be replaced by complementary colors, e. g. blue by yellow.

Some asymmetry of the patterns was also recognized: With the azimuth of 0° or 90° of the polarizer, the bands in the temporo-inferior and naso-superior quadrants were in

Fig. 7. The crossing fibers of the cornea in the central region, the azimuth of the polarizer; 0° or 90°.



majority of cases more numerous (the order of colored bands unchangdd and repeatedly) than those in naso-inferior and temporo-superior quadrants respectively. This phenomenon might possibly be due to some asymmetrical structure of the cornea.

Fibers of Cornea

The central region. The central region of the cornea revealed dark fibers which intersected approximately at a right angle with each other, as illustrated in Fig. 7. For the observation of these fibers, dilation of the pupil was needed. The retro-illumination method and the magnification of 25× or 40× were found to be adequate.

Even if the corneal epithelium was removed, these fibers remained unchanged in their appearance. Therefore, these fibers are concluded to be the structure of the corneal stroma.

With the azimuth of 0° or 90° of the polarizer, these crossing fibers appeared to run obliquely at an angle of approximately 45° to the direction of the polarizer and the analyser respectively. When both polars were rotated, they were also rotated in the same direction and to an equal extent; with the azimuth of 45° or 135° they run vertically and horizontally.

In the central region, therefore, when the polarizer and the analyser are rotated adequately, the crossing fibers running in any direction can be observed.

The peripheral region

a) the crossing fibers. The crossing fibers, running in the same direction as those in the central region were distinctly visible in the peripheral region at the site of the arms of the hyperbolic figures. These fibers extended to the superficial layer of the limbus. It was also possible to get some evidences in favor of the opinion that these crossing fibers were situated mainly in the superficial layer.

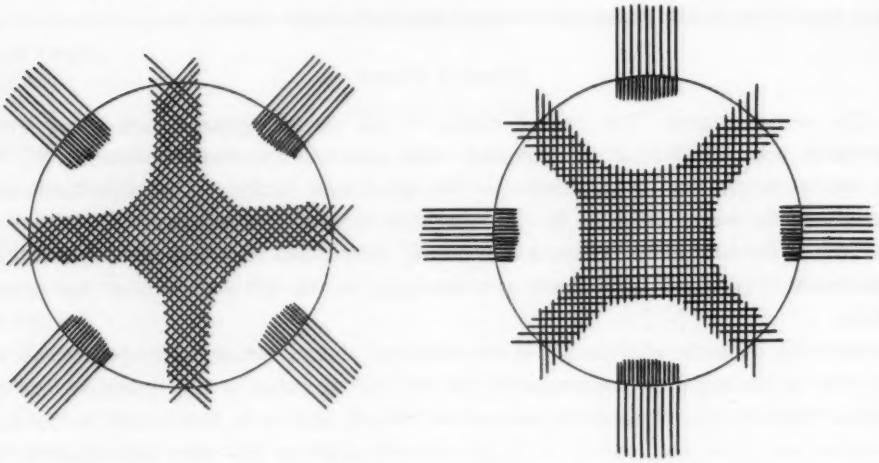
In the bright region diagonal to the arms of the hyperbolic figures, only a few fibers were visible. But in the superficial layer of both the extreme periphery of the cornea and the limbus, fibers running in a radial manner were abundant and continued to the subconjunctival tissue. These fibers appeared to be the same in nature as crossing fibers of the peripheral cornea.

b) the radial fibers. In the deep layer of the bright and colored region, diagonal to the arms of the hyperbolic figures, fibers of another type can be observed with high magnification in some favorable cases. These fibers, which run radially, appeared closely aggregated and faintly tinted. They also could be seen with parallel polars.

The factors influencing the visibility of the fibers. The visibility of the fibers showed individual variation to some extent. Although the crossing fibers were constantly visible in normal cornea, the observation of the radial fibers was often very difficult.

The sort of the background, namely either the lens or the iris, the magnitude of the illumination-observation angle, the direction of observation and the version of the eyes also had serious influences on it. Hence in order to visualize the fibers constantly, these

Fig. 8. Schematic illustrations of the patterns of the appearance of corneal fibers, the azimuth of the polarizer; a) 0° or 90°, and b) 45° or 135°.



factors should be standardized as described above.

The arrangement of corneal fibers. The schematic illustrations of the appearances of fibers are illustrated in Fig. 8 a and 8 b. Judging from these appearances, a characteristic arrangement of corneal fibers may be assumed: In the peripheral region, radial fibers predominate in the deep layer, while the central cornea and the superficial layer of the peripheral cornea are composed of crossing fibers in random arrangement.

Comment

Cogan[1] described first the dark cross-shaped figure of the living human cornea. He could not, however, find out the point of intersection of the cross-figure due to the red reflex from the pupil. He postulated that the appearance of the cross-figure was attributed to the radial arrangement ot the elements in the cornea, although no anatomical evidence in favor of his opinion had been reported.

Stanworth and Naylor[3] paid their particular attention to the appearance of the cross-shaped and hyperbolic interference figures in the isolated cornea placed between crossed polars. They also established a schema representing the principal direction of vibration of rays passing through the cornea, and showed that the retardation of rays normal to its surface is the minimum in the center, increasing toward the periphery.

Koeppe[5] observed dark crossing lines of the living human cornea and postulated them to be a cement substance connecting corneal lamellae. He could not, however, put forward the pattern of the arrangement of the lines.

In the isolated cornea of rabbits, Kikkawa[2][3][4] reported that the fibers, which are composed of fringed biaxial micelles, have characteristics of two-dimensional extension. According to his description, the optical property of the polarization of the fibers is such that the Z-axis is at right angles to the direction of fibers, the Y-axis is parallel thereto, and the X-axis is normal to the plane of the cornea. As for the arrangement of the

fibers, he confirmed that the fibers in the deep layer are chiefly arranged radially in the peripheral region and intersect with each other in the central region, while in the superficial layer, they show an appearance of closely intersecting meshwork.

It is worth particular attention that the arrangement of the human corneal fibers described above is in good accordance with Kikkawa's report on the rabbits cornea. Accordingly, it may be reasonable to conclude that the fibers observed in the living human cornea are the same in nature as that observed in the isolated cornea with a polarizing microscope, although it is extremely difficult to give an adequate optical explanation to their appearance. If this view is valid, the visibility of fibers composed of fringed micelles should be much changed by an application of stretch, in consequence of the rotation of the principal direction of vibration. In fact, fibers of a living rabbit cornea disappeared by stretching and reappeared by relaxation. This fact also supports the conclusion mentioned above.

The so-called dark cross is composed of a set of hyperbolic figures, as described by Stanworth and Naylor in the isolated cornea. Therefore, the author is of the opinion that the term "the dark cross" should be substituted for "the dark hyperbola". The dark hyperpola observed with the optical section suggests that this figure has an intimate relation to the deep layer of the cornea. Judging from the arrangement of fibers of cornea, it may be said that this figure is a representative of the radial arrangement of the fibers which predominate at its deep layer, as predicted by Cogan.

The colored bands, which are seen on the surface of the iris, are also attributable to the interference phenomenon due to the double refraction of rays passing through the cornea. In the region, diagonal to the arms of the hyperbolic figures, the fibers in the deep layer run at an angle approximately 45° against the direction of the polarizer. Therefore, the rays passing through this region will undergo larger retardation, the magnitude of which determines the color. The oblique incidence of light and the increased thickness of the peripheral cornea, as considered by Stanworth and Naylor, also increase the the magnitude of retardation.

At any rate, the characteristic patterns of the dark hyperbola, the interference color and the corneal fibers as well can be constantly observed with ease by means of the biomicroscope equipped with polars. This technique, therefore, may be quite useful for the study of pathological conditions of the cornea in situ. Further details of this technique for clinical application and the interpretation of pathological findings will be published elsewhere.

Summary

A new technique of the biomicroscopy with polarized light has been introduced. By means of this technique, the structure of the normal human cornea was studied with following results:

1. The so-called dark cross of the cornea is not a complete cross, but is composed of a set ot hyperbolic figures. Therefore, the term "the dark hyperbola" was proposed by

the present author. The factors influencing the shape and the site of the figures were discussed.

2. The hyperbolic figures appeared as a dark gap in the optical section. It was concluded that the figures are intimately related to the structure of the deep layer of the cornea, in which radial fibers predominate.

3. The colored bands in multilayers, which appeared to lie on the surface of iris, were attributed to the interference phenomenon due to the double refraction of rays passing through the cornea.

4. The fibers could be easily observed in the living human cornea. The nature of these fibers was discussed. The characteristic arrangement of the fibers was as follows: In the peripheral cornea, fibers of the deep layer are arranged chiefly in a radial manner, while they are intersected with each other in the superficial layer. On the contrary, fibers in the central region are intersected with each other throughout the whole layer.

(Rearranged from 3 articles published in Japanese in Acta. Ophth. Jap. 61:461-465, 1957, 62:380-384, and 492-497, 1958.)

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PHOSPHATASE ACTIVITIES IN THE OCULAR TISSUE

Changes in Activities of ATP-ase and 5-Nucleotidase of Visual Cells by Light and Dark Adaptation

Yoshinao MAJIMA

Department of Ophthalmology, School of Medicine, Nagoya University

De Concillisis was the first to report on the presence of phosphatase in the retina, while J.L. Reis found a enzyme that acts on muscle adenylic acid, and is termed 5-nucleotidase, and from its relationship with phosphomonoesterase pointed out the specificity of this enzyme. Regarding the histologic picture of phosphatase in various parts of the retina, there are the reports of histochemical studies made by K. Kojima et al[5]. and S. Hayano[4]. Recently Y. Majima[8]. reported on the dynamic variation of acid and alkaline phosphatase and ATP-ase in the retine, by light and dark exposures. K. Ezuka[3] found

energy rich phosphate bonds to be present in the retina, that the amount of ATP fell during light exposure, while the activity of ATP-ase became enhanced. From these findings he suggested that light adaptation stimulates the so-called phosphate metabolism, and that this play the role of initiating the metabolism of the retina. However, regarding the enzymatic observations of the visual cells that known to produce with photochemical changes, there is only the report of Y. Majima[9] on phosphomonoesterase, and no studies have yet been made of the actions of phosphatase in the visual cells on the adenylic acid compounds. In this paper studies were made of the activities of ATP-ase and 5-nucleotidase in the retina and its visual cells, by light and dark adaptation.

Experimental Method

Measurement of ATP-ase activity

a) Du Bois and Potter's[2] method was employed.. For a warm blooded animal optimal temperature, 37°C.

b) Henry A Lardy and Harlene Wellman's[7] method was employed.

For the frog retina optimal temperature, 25°C.

Measurement of 5-nucleotidase. With the object of studying under a condition identical with that for phosphomonoesterase, Shinowara-Jones- Reinhart's[12] method was employed, while as the substrate muscle adenylic acid, a product of N.B.C. Co. was used.

Experimental Materials

Bovine, rabbit, and frog retina. The frog retinas were separated into two parts, one part with the outer segment of the rods and the other part consisting of the remaining retina removed according to Z. Saito's[11] method.

Results

1) Table 1, a). At pH. 7.4 the activity of retinal ATP-ase was 2-2.5 times that of non-specific phosphatase.

2) Table 1, b). At pH. 7.4 the activity of 5-nucleotidase was 6-7 times that of non-specific phosphatase.

Table 1. (a)
Phosphomonoesterase and ATP-ase
(Bovine retina)

Phosphomonoesterase (pH 7.4)	0.12±0.03
ATP-ase (pH 7.4)	0.31±0.06

Unit: P mg/g/15 min. (Fresh weight)

Table 1. (b)
Phosphomonoesterase and 5-Nucleotidase
(Rabbit retina)

Phosphomonoesterase (pH 7.4)	0.14±0.05
5-Nucleotidase (pH 7.4)	0.90±0.11

Unit: P mg/g/h. (Fresh weight)

Table 2.
Changes in Activities of the various Phosphatases of the Frog Retina by Light and Dark Adaptation

Tissues	Visual cell		remaining portions of the retina	
Conditions / Kinds of enzyme	light-adaptation	dark-adaptation	light-adaptation	dark-adaptation
1) Phosphomonoesterase				
a) Acid Phosphatase (pH. 5.0)	4.05±1.25	3.35±0.95	1.40±0.35	1.05±0.20
b) Neut. Phosphatase (pH. 7.4)	0.50±0.05	0.49±0.11	1.07±0.15	0.86±0.18
c) Alkali Phosphatase (pH. 10.9)	2.10±1.1	1.70±0.70	0.85±0.11	0.80±0.16
2) ATP-ase (pH. 7.4)	0.61±0.20	0.86±0.18	1.05±0.11	0.81±0.17
3) 5-Nucleotidase (pH. 7.4)	1.35±0.41	0.74±0.27	2.29±0.30	1.26±0.39

Unit: P γ/mg/h (PM-ase, 5-Nucleotidase) (Dry Weight)
Unit: Pγ/mg/10min. (ATP-ase) (Dry Weight)

3) Table 2 & Fig. 1. Of the various of phosphatase in the retina, acid phosphatase was most active in the visual cells, while the activity of phosphatase was lowest at pH. 7.4. Almost all the phosphatase in the visual cell showed a tendency for activity to rise by light exposure, the activity of 5-nucleotidase being especially marked, with the activity by light adaptation rising to 1.5 times that by dark adaptation. Against these, only ATP-ase tended to rise somewhat during dark adaptation.

4) Table 2 & Fig. 2. With the remaining part of the retina excluding the visual cells, there was not seen a difference as great as in the case of visual cells, in the activities, of, neutral and alkaline phosphatase between light and dark adaptation. And as was noted above, the activity of 5-nucleotidase during light adaptation rose to 1.5 times that during dark adaptation. This fact would seem to indicate that this enzyme is somewhat connected with the mechanism of production of phosphoric acid brought about by light exposure. In the case of ATP-ase, contrary to that seen in the visual cells, light exposure tended to increase the activity.

Fig. 1. Changes in activities of the various phosphatases of visual cell (mainly of the outer segment of rods) by light and dark adaptation.

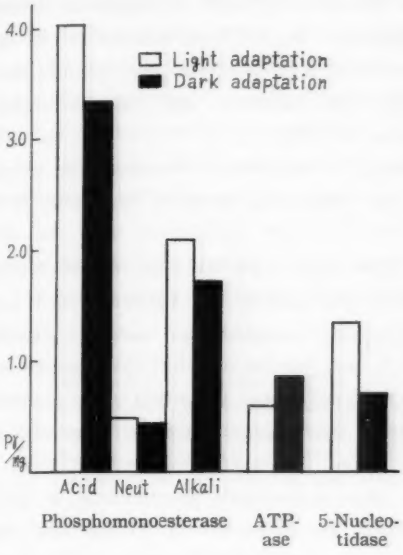
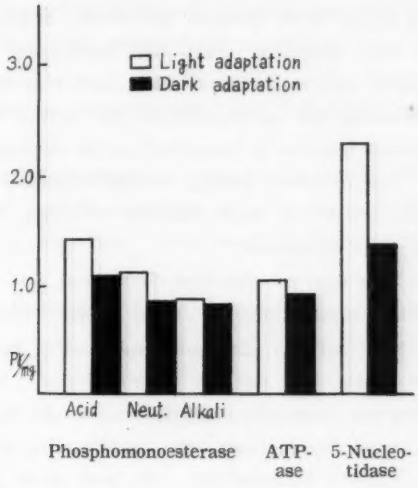


Fig. 2. Changes in activities of the various phosphatases of remaining part of the retina by light and dark adaptaton.



Consideration

ATP-ase activity in the retina. Changes in activity of ATP-ase of visual cells (mainly of the outer segment of rods) and the remaining parts of the retina by light and dark adaptation.

It has been reported by Y. Majima that ATP-ase exists in the retina, but from a comparison of this enzyme with energy-rich phosphate bonds, and phosphomonoesterase, it was noted that under the same conditions the activtiy of ATP-ase rises to 1.5-2 times. Regarding the activity of this enzyme there has been the report of K. Ezuka, but his observations were made of the whole retina. Hence in this report, estimations were made especially of the changes in this enzyme by light and dark adaptation, in the visual cells (mainly of the outer segment of rods), which are the existence of photosensitive substance. It was found that the activity of ATP-ase of visual cells tends to rise during dark rather than light adaptation, while in remaininy parts of the retina excluding the visual cells a contrary tendency was noted, and the activity rose during light adaptation. Needless to say, during both light and dark adaptation, ATP-ase is instrumental in initiating phosphorylation and plays an important role in the various metabolic processes in the retina.

In the visual cells, especially the outer segment of rods, the activity of ATP-ase is seen to rise during dark adaptation, resulting in the decomposition of ATP, while it has been pointed out by Collins[1] F.D. and Y. Koide[6] that ATP and these energy rich

phosphate bonds act as sources of energy for the regeneration of visual purple. Thus it would seem that these substances are not only energy sources of metabolism but that the surplus energy is utilized intentionaly in the regeneration of visual purple. Against this in the other parts of the retina, despite no effects being noted in phosphomonoesterase by light exposure, there has been noted a tendency for ATP-ase activity to rise by K. Ezuka[3] and S. Tanaka[13] et al. and it is believed that ATP-ase holds high significance in promoting the metabolism of the retina during light exposure, and the conduction on nervous impulse is concerned in the decomposition of ATP.

5-nucleotidase activity in the retina. Changes in activity of 5-nucleotidase of visual cells (mainly of outer segment of rods) and the remaining parts of the retina by light and dark adaptation

This enzyme was first discovered by J.L. Reis who reported that in the retina of most animals there is found besides non-specific phosphatase, an enzyme with a higher grade of activity that acts specifically on adenosine-5-phosphate and inosine-5-phosphate, and called this enzyme 5-nucleotidase. Later it was pointed out that this enzyme is not necessary a specific phosphatase, but in 1951, J.L. Reis[19] stated again that under physiologic hydrogen ion concentration condition the activity of this enzyme becomes for greater than non-specific phosphatase. As was made clear from the experiments reported here, this enzyme was found to be 6-7 times more active than non-specific phosphatase at pH 7.4, and this enzyme which exhibits a high grade of specificity in the retina was closely related to the metabolism of the adenylic acid compounds plays the role of the starter in the metabolic processes of the retina, especially of respiration and glycolysis. In this report therefore, such an observation was attempted, and under physiologic hydrogen ion concentration conditions comparisons were made with the 5-nucleotidase and the other phosphatases, and it was found that in both the visual cells and remaining parts of retina the activity of 5-nucleotidase is highest, while by light exposure the activity was raised to about 1.5 times. This is believed to be closely related to the production of phorphoric acid in the retina by light adaptation, but the details regarding this have to await further investigations. At any rate, it is extremely interesting that this enzyme which is believed to be specific for the retina presented such results.

Conclusions.

Under physiologic hydrogen ion concentration conditions the activities of retinal specific phosphatase (5-nucleotidase), non-specific phosphatase, and ATP-ase were compared. The effects on these enzymes brought about by light and dark adaptations were also investigated in visual cells and remaining parts of the retina.

1. *Changes in ATP-ase activity*

a) The activity of ATP-ase in bovine retina measured by means of Du Bois and Potter's method was about 2-2.5 times higher than that for non-specific phosphatase.

b) The retina of frog was separated into the part consisting of visual cells (mainly

of outer segment of rods) and remaining part, and the activity of ATP-ase was examined according to Lardy and Wellman's method during dark and light adaptation. It was then found that the activity in the visual cells tended to rise during dark rather than light adaptation, while in the remaining part of the retina a contrary phenomenon was noted and the activity tended to rise during light rather than during dark adaptation.

2. *Changes in 5-Nucleotidase activity*

a) The activity of 5-nucleotidase measured in rabbit retina, was 6-7 times higher than that for non-specific phosphatase.

b) The frog retina was separated into a part consisting of visual cells (mainly of the outer segment of rods) and the remaining part, and the activities of this enzyme during light and dark adaptation were estimated. It was then found that in both parts the activity rose to 1.5 times by light exposure.

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VITAMIN A CONTENT IN LIVER AND B_1 CONCENTRATION IN BLOOD OF MICE MAINTAINED ON VITAMIN A OR B_1 DEFICIENT DIET

Toshikazu IDE and Masashi SHIMO-OKU
Department of Ophthalmology, Kobe Medical College

Introduction

Many authors have investigated the effect of certain vitamin to eye diseases and its correlation to another kind of vitamins. Some concluded, that the vitamin B_1 content in the blood of vitamin A deficient mice is also reduced. In the present paper, the vitamin A content of the liver and the vitamin B_1 concentration in the blood of mice which were fed either with vitamin A or vitamin B_1 deficient diet were studied, so as to serve for the basic experiments to the etiologic inquiries into the retrobulbar neuritis—a subject much investigated at our department by Prof. Jo Imachi and his coworkers.

Experimental Method

Healthy male mice, body weight 15-25 g., were divided into three groups. Each group was fed respectively on normal, vitamin A or B_1 deficient diet (see the headings of table 1).

Table 1. Composition of Diets (one day's feed for a mouse)

Diet / Ingredients	Normal diet	V.A deficient diet	V.B_1 deficient diet
1. Cleaned rice flour	1.78	1.78	1.78
2. Purified casein	0.9	0.9	0.9
3. Ebios	0.16	0.16	0
4. Mixed salts	0.12	0.12	0.12
5. Cod liver oil	0.06	0	0.06
6. Water	Some	Some	Some
V.A content	464 I.U.	0	464 I.U.
V.B_1 content	95.5 γ%	95.5 γ%	38.3 γ%

The bodily conditions were carefully observed and vitamin A in the liver and B_1 in the blood were quantitatively estimated at every 10 days. On the experimental day the blood was drawn from the heart, and vitamin B_1 was ascertained fluorometrically by the cyochrom method, using 0.5 cc. of blood. The vitamin A contents in the liver were measured at the same time by the glycerol dichlorhydrin method.

Results

1) General condition of vitamin deficient mice

Vitamin A deficiency. Body weight of the vitamin A deficient mice decreased gradually and in many cases the hair became coarse and dirty, but the pathological symptoms typical for vitamin A deficiency such as xerophthalmos and keratomalacia were not observed. The mortality in the course of experiment reached 20-30%.

Vitamin B_1 deficiency. In the vitamin B_1 deficient diet body weight markedly decreased, and on the 30th or 40th day from the commencement of the experiment paralysis and tremor of both extremities, ataxia and soiled hair were observed. On the 45th day the mortality reached 30-40%.

2) Chemical data

The vitamin contents in the tables were statistically evaluated.

Vitamin A content. in the vitamin A deficient liver. The vitamin A contents of the successive experimental days were 404, 276, 199 and 216 I.U.. Thus there is a heavy drop, roughly 33%, in the 2nd experimental day from the 1st, and again some 28% drop between the 2nd and the 3rd. But later the A-level seemed to be settled and we have

the 3rd and the last practically unchanged.

Vitamin B_1 concentration in the vitamin A deficient blood. The total vitamin B_1 concentration in the blood of the successive experimental days were 17.1, 16.9, 16.2, 16.3 and 15.7γ%. The free vitamin B_1 level was 6.3, 5.8, 5.5, 5.5 and 5.2γ%. Thus, the vitamin B_1 level in the blood of this diet showed a gradual diminution in the course of the deficiency, i. e. 9% drop in the total content and 17% drop in the free fraction on the 50th experimental day.

Table 2. Vitamin A Content in the Liver on Every Experimental Day (I.U/g)

Exp. day	Control group		V.A deficient group		V.B_1 deficient group	
	Number of mice	Mean	Number of mice	Mean	Number of mice	Mean
10	6	470±245	9	404±134	10	393±118
20	6	748± 68	7	276±44	9	1178±129
30	7	1774±299	10	199±53	9	1003±104
40	7	2833±219	6	216±67	10	1179±258

Table 3. Vitamin B_1 Concentration in the Blood on Every Experimental Day. The Content of Free Fraction is shown in the Parentheses (γ%)

V.A deficiency			V.B_1 deficiency		
Exp. day	Number of mice	Mean	Exp. day	Number of mice	Mean
Control	5	17.2±0.5	Control	5	17.2±0.5
	(5)	(7.3±0.2)		(5)	(7.3±0.2)
10	4	17.1±0.5	10	5	16.6±0.6
	(4)	(6.3±0.9)		(5)	(6.0±0.8)
20	5	16.9±0.6	20	5	16.5±0.9
	(5)	(5.8±0.2)		(5)	(5.8±0.2)
30	5	16.2±0.6	30	5	14.5±1.2
	(5)	(5.5±0.8)		(5)	(4.6±0.4)
40	5	16.3±1.0	40	5	11.9±1.0
	(5)	(5.5±0.8)		(5)	(4.6±0.2)
50	4	15.7±0.1	45	4	12.0±0.6
	(4)	(5.2±0.2)		(3)	(3.6±0.2)

Vitamin A content in the vitamin B_1 deficient liver. It was observed that the vitamin A content in the liver increased in accordance with the course of the vitamin B_1 deficiency and on the 20th vitamin B_1 deficient day it reached at 1178 I. U., and thereafter, in spite of the progress of the vitamin B_1 deficiency, the vitamin A content did not show any marked variation.

Vitamin B_1 concentration in the vitamin B_1 deficient blood. The total vitamin B_1 concentration in the blood of every experimental day were 16.6, 16.5, 14.5, 11.9 and 12.0γ%, and the free vitamin B_1 levels were 6.0, 5.8, 4.6, 4.6 and 3.6γ%. Thus it was shown that total and free vitamin B_1 concentration in the blood markedly decreased in due course of vitamin B_1 deficiency. When the vitamin B_1 concentrations in the vitamin B_1 deficient blood compared with those of vitamin A deficient blood, it was observed that on the 20th experimental day, there was no remarkable difference between them, but on the 30th experimental day, the former decreased more markedly than the latter.

Discussion

In the above experiments the typical symptoms of vitamin A deficiency (keratomalacia and xerophthalmos) were not observed in mice.

With regard to the correlation between vitamin A and B_1 deficiency, some authors recognized that there is no correlation at all, and others reported that there is an antagonistic action between them, and while still others insisted on the presence of a synergic action. For example, Imachi, in an experiment using rats and rabbits, showed that in vitamin A deficient condition the vitamin B_1 concentration in the blood decreased to 50-60% of the normal level, and in vitamin B_1 deficiency, the similar vitamin A content was also reduced to 60% of the normal, and suggested a probable action of the thyroid gland being responsible for the mechanism. Nagahama observed that vitamin B_1 affected indirectly the metabolism of vitamin A by regulating the function of the reticuloendotheliar system in the liver.

Shimo-oku, one of the present authors, described histopathological changes in the liver fed with a diet similar to that used above—the changes observed were atrophies of liver cells, a sort of fatty degeneration of lobuli, especially in the periphery and round cell infiltrations in the perivascular spaces of interlobular tissues and the columns of liver cell, so that one may assume the decrease either in vitamin A or B_1 content found here to be highly correlated to these changes in the liver.

Summary

Vitamin A content in the liver and B_1 concentration in the blood of the mice fed with vitamin A or B_1 deficient diet were measured and following results were obtained.

1. The vitamin A content in the liver was 216 I. U. on the 40th vitamin A deficient day. The total vitamin B_1 concentration in the blood was 15.7γ%, and the free fraction was 5.2γ% on the 50th vitamin A deficient day, showing the vitamin B_1 concentration in

the blood maintained on the vitamin A deficient diet clearly decreased as compared with the normal level.

2. The total vitamin B_1 concentration in the blood fed with vitamin B_1 deficient diet was 12.0γ% on the 45th deficient day and the free vitamin B_1 was 3.6γ%. Vitamin A content in the liver fed with this diet was 1179 I. U. on the 40th deficient day and moderately lower than the normal value.

3. Both the vitamin B_1 concentration in the blood fed with the vitamin A deficient diet and the vitamin A content in the liver fed with the vitamin B_1 deficient diet decreased in accordance with the lapse of the experimental day.

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THE EFFECT OF ELECTRICAL STIMULATION OF THE HYPOTHALAMUS ON THE TISSUE RESPIRATION OF THE IRIDOCILIARY BODY AND ON THE PROTEIN CONTENT IN AQUEOUS HUMOR

Aiko FUKASAWA

Department of Ophthalmology, School of Medicine, Kanazawa University

The study of the relation between the hypothalamus, respectively Kurotsu's sympathetic or parasympathetic zones and eye have been reported by Nagai that the intra-ocular pressure and the protein content in the aqueous humor were changed by the stimulation of the sympathetic zone, but there showed no remarkable change by the stimulation of the parasympathetic zone except the decrease of the intra-ocular pressure. The changes of the eye caused by the electrical stimulation of the hypothalamus would be thought as one of Selye's alarm reaction.

The author has studied the effect of the electrical stimulation of Kurotsu's c-parasympathetic and b-sympathetic zones of rabbits on the tissue respiration of the iridociliary body and the protein content in the aqueous humor.

Methods

The stimulation of the hypothalamus was followed by the Kurotsu's method. Stimulating current was obtained from the thyratron regulated discharge of 0.1 mfd condenser, with a time constant of 1.4 msec.. For routine exploration a stimulus intensity of 12 volts and 60 per second was commonly used. Electrical stimulation for one minute with 30 seconds pause was repeated 10 times.

The tissue respiration of the iridociliary body was measured by the Warburg's indirect

method.

The protein nitrogen and the non protein nitrogen were measured by the micro-Kjeldahl's method and nesslerization.

Results

1) The change of the general state followed by electrical stimulation

Stimulation of the b-sympathetic zone. Extreme dilatation of pupil, exophthalmos, palpitation, tachycardia etc. appeared and sometimes petechiae in the auricle. These symptoms would be thought of as a state of sympathicotonia.

Stimulation of the c-parasympathetic zone. Miosis, moderate dilatation of the auricle vessels, bradycardia, enophthalmos etc. were seen.

2) The change of tissue respiration of the iridociliary body is shown in Table I, II, III and IV.

Stimulation of the sympathetic zone. The value of Qo_2 decreased after the stimulation, 19% in one hour and 30% in two hours after the stimulation. This change recovered in six hours or so. There showed no remarkable change in the aerobic glycolysis. The

Table I. The Changes of the Tissue Respiration of the Iridociliary Body by the Electrical Stimulation of the Sympathetic Zone

	control		immediately after stimul.		30 minutes after stimul.		1 hour after stimul.		2 hours after stimul.		6 hours after stimul.	
No.	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$
1	−4.1	+0.4	−4.0	+0.4	−2.5	+1.8	−2.8	0	−3.0	+0.6	−2.7	+0.4
2	−4.2	0	−5.4	+0.5	−5.3	+1.5	−3.4	+0.9	−4.0	+0.6	−4.2	+0.5
3	−4.0	+0.8	−1.5	0	−4.0	+0.5	−3.7	+0.6	−2.4	0	−4.4	+0.8
4	−4.4	+0.4	−3.9	+0.2	−4.6	+0.8	−3.0	+0.3	−3.1	+0.5	−4.1	+0.4
5	−4.9	+0.5	−4.5	+0.1	−3.1	0	−3.5	+0.2	−2.7	0	−3.9	0
6	−4.1	0	−3.4	0	−4.1	0	−4.7	+0.4	−3.2	+0.2	−4.4	+0.3
Average	−4.3	+0.4	−3.7	+0.2	−3.9	+0.8	−3.5	+0.4	−3.0	+0.3	−4.0	+0.4

Table II. The Changes of the Tissue Respiration of the Iridociliary Body by the Electrical Stimulation of the Parasympathetic Zone

	control		immediately after stimul.		30 minutes after stimul.		1 hour after stimul.		2 hours after stimul.		6 hours after stimul.	
No.	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$	Qo_2	$Q_M^{O_2}$
1	−6.4	+0.3	−4.7	+0.6	−4.3	0	−4.6	0	−5.3	0	−5.9	+0.8
2	−5.0	+0.2	−5.5	+0.8	−3.0	0	−3.9	+0.5	−4.2	0	−4.1	+0.3
3	−5.2	+0.4	−3.8	0	−3.4	0	−5.2	0	−4.0	0	−4.8	0
4	−4.4	+0.2	−3.7	0	−4.7	0	−3.7	+0.2	−6.3	0	−3.3	0
5	−3.6	0	−5.8	0	−6.6	+0.7	−4.2	0	−3.8	0	−4.9	0
6	−4.9	0	−4.5	0	−5.5	+0.2	−4.5	0	−4.9	0	−4.6	0
Average	−4.8	+0.2	−4.6	+0.2	−4.6	+0.2	−4.4	+0.1	−4.8	0	−4.6	+0.2

Table III. The Change of the Anaerobic Glycolysis of the Iridociliary Body by the Electrical Stimulation of the Sympathetic Zone

No.	control	No.	immediately after stimul.	No.	30 minutes after stimul.	No.	1 hour after stimul.	No.	2 hours after stimul.	No.	6 hours after stimul.
1	+13.2	9	+12.1	17	+11.8	25	+10.5	33	+10.7	41	+12.9
2	+12.0	19	+12.0	18	+11.5	26	+10.2	34	+11.0	42	+12.3
3	+13.7	11	+11.9	19	+10.6	27	+11.1	35	+10.8	43	+13.5
4	+13.6	12	+11.9	20	+11.4	28	+10.2	36	+ 9.6	44	+13.0
5	+13.4	13	+12.3	21	+10.9	29	+ 9.8	37	+ 9.3	45	+12.8
6	+13.0	14	+12.2	22	+11.2	30	+ 9.9	38	+10.0	46	+12.6
7	+14.2	15	+10.7	23	+11.2	31	+10.3	39	+ 9.8	47	+13.4
8	+12.7	16	+10.9	24	+11.5	32	+10.4	40	+10.4	48	+13.1
Average	+12.8		+11.8		+11.2		+10.3		+10.2		+13.0

Table IV. The Changes of the Anaerobic Glycolysis of the Iridociliary Body by the Electrical Stimulation of the Parasympathetic Zone

No.	control	No.	immediately after stimul.	No.	30 minutes after stimul.	No.	1 hour after stimul.	No.	2 hours after stimul.	No.	6 hours after stimul.
1	+13.2	9	+13.7	17	+12.6	25	+13.4	33	+11.3	41	+13.2
2	+12.0	10	+12.6	18	+10.8	26	+13.0	34	+12.3	42	+13.0
3	+13.7	11	+12.1	19	+11.5	27	+12.4	35	+11.9	43	+12.8
4	+13.6	12	+10.8	20	+12.8	28	+11.5	36	+10.8	44	+12.9
5	+13.4	13	+13.7	21	+13.4	29	+12.9	37	+11.4	45	+11.8
6	+13.0	14	+14.7	22	+11.2	30	+12.1	38	+12.8	46	+12.8
7	+14.2	15	+12.1	23	+11.7	31	+10.8	39	+13.0	47	+12.5
8	+12.7	16	+10.5	24	+12.1	32	+11.8	40	+13.4	48	+12.4
Average	+12.8		+12.6		+12.1		+12.2		+12.1		+12.7

Table V. The Changes of Total Nitrogen Content in the Aqueous Humor by the Electrical Stimulation of the Sympathetic Zone (mg. N/dl.)

No.	control	No.	immediately after stimul.	No.	30 mitnutes after stimul.	No.	1 hour after stimul.	No.	2 hours after stimul.	No.	6 hours after stimul.
1	44.0	4	56.2	7	75.0	10	125.0	13	52.6	16	42.6
2	40.0	5	45.0	8	53.8	11	88.8	14	85.0	17	51.2
3	43.8	6	48.6	9	66.0	12	71.2	15	85.0	18	44.4
Average	42.6		50.0		65.0		95.0		74.2		49.2

Table VI. The Changes of Non Protein and Protein Nitrogen Content in the Aqueous Humor by the Electrical Stimulation of the Sympathetic Zone (mg. N/dl.)

	No.	Total N	No.	non protein N	Protein N
control	1	43.8	10	37.8	6.0
	2	38.0	11	32.8	5.2
	3	39.0	12	34.2	5.0
	Average	40.4		35.0	5.4
2 hours after stimul.	4	42.6	13	47.6	35.0
	5	67.6	15	28.8	38.8
	6	82.4	14	35.5	46.9
	Average	64.2		37.3	40.2
2 hours after stimul.	7	37.2	16	31.0	6.2
	8	41.2	17	29.2	12.0
	9	43.6	18	36.3	7.3
	Average	40.7		32.2	8.5

Table VII. The Changes of Non Protein and Protein Nitrogen Content in the Aqueous Humor by the Electical Stimulation of the Para-Sympathetic Zone (mg. N/dl.)

	No.	Total N	No.	non protein N	Protein N
control	1	42.4	10	36.2	6.2
	2	42.8	11	37.0	5.8
	3	40.2	12	35.3	4.9
	Average	41.8		36.2	5.6
2 hours after stimul.	4	42.3	13	34.8	8.5
	5	43.8	14	34.0	9.8
	6	41.5	15	33.2	8.3
	Average	42.9		34.0	8.9
6 hours after stimul.	7	45.0	16	34.3	10.7
	8	42.2	17	35.6	6.6
	9	40.8	18	34.3	6.5
	Average	42.7		34.7	7.9

anaerobic glycolysis showed about 20% decrease in one or two hours and recovered in six hours or so after the stimulation.

Stimulation of the parasympathetic zone. The value of Qo_2 decreased by 8.3% in one hour after the stimulation. There showed no remarkable change in other cases. The aerobic glycolysis ($Q_M^{O_2}$) showed no remarkable change and the anaerobic glycolysis ($Q_M^{N_2}$) showed a slight decrease in one or two hours.

3) The change of nitrogen content in the aqueous humor is shown in Table V, VI and VII.

Stimulation of the sympathetic zone. The quantity of protein nitrogen was 7.4 times greater than the normal value in two hours after the electrical stimulation and came near to the normal quantity in six hours. The non protein nitrogen content showed no remarkable change.

Stimulation of the parasympathetic zone. The protein nitrogen content showed a 1.5 times increase in two hours and a 1.3 times in six hours after the stimulation, but the non protein nitrogen showed a slight decrease.

Comment

In the theories of olden times it was maintained that several centers of the specific autonomic function exist separately in the hypothalamus, but recently Ochi and Wataru, Beattie, Hess, Ranson, and Kurotsu indicated that the sympathetic and parasympathetic centers exist in the hypothalamus as a whole. Kurotsu reported that there were three kinds of areas a-parasympathetic, b-sympathetic, c-parasympathetic zones. He and his associates reported, that the rise of the intra-ocular pressure, the increase of the protein in the aqueous humor, the increase of the transfer of uranin and the histological changes in the iridociliary body were shown by the electrical stimulation of Kurotsu's b-sympathetic zone. By my experiments the metabolism of the iridociliary body was also changed by the electrical stimulation of the zone. The Qo_2 of iridociliary body showed 14% decrease in 30 minutes, 19% decrease in one hour and 30% decrease in 2 hours after the stimulation, but the $Q_M^{O_2}$ showed very slight increase.

On the other hand, similary to Takagi's and Takemura's the author observed significant hyperemia and edema in the iridociliary body, especially in the processus ciliare. Furthermore the author observed the appearance of fibrin and sometimes the detachement of the epithel cells of the ciliary body.

It is easily assumed that these above mentioned tissue changes will bring the changes of the intracellular structure and naturally the tissue metabolism, which is very sensitive to the intracellular structure.

It is evident that the increase of total nitrogen in the aqueous humor after stimulation of the b-sympathetic zone depends mostly upon the increase of protein nitrogen. The aqueous humor is produced by the specific activity of the ciliary body, and the equilibrium between the protein content of the aqueous humor and of the serum depends upon the function of it. So the increase of protein content in the aqueous humor caused by the electrical stimulation of the b-sympathetic zone is certainly due to the transfer of serum protein into the anterior chamber, which resulted from the change of the permeability of of the ciliary epithel.

Concerning the mechanism of the damage of the ciliary body, two factors shall be considered. One of them shall be the prompt rise of blood pressure and the contraction of capillary in the ciliary body as Takagi and Takemura reported. Another shall be one of the symptomes of the anti-shock stadium of the alarm reaction, in which fibrinolysin

is activated by adrenocortic hormon or destruction of cells of organs and the change of the membrane permiability is caused. But the certification of this assumption takes more study.

Secondly Kurotsu and his associates reported that hypotension, the decrease of intraocular pressure, normal or retardative transferrence of uranin and a very slight change of nitrogen content of the aqueous humor were observed by the electrical stimulation of the c-parasympathetic zone. By my experiments, in respect of the tissue respiration, Qo_2 showed a slight decrease and $Q_M^{O_2}$ no remarkable change and furthermore $Q_M^{N_2}$ showed a slight decrease. Also the change of total nitrogen and protein nitrogen in the aqueous humor caused by the stimulation of its zone were very smaller than that caused by the sympathetic zone stimulation. The histological changes (hyperemia and dilatation of capillary) were very slight, too. So it seemed, that the mechanism of the production of aqueous humor was nearly not disturbed by the parasympathetic stimulation.

Summary

Stimulating electrically the Kurotsu's b-sympathetic and c-parasympathetic zone of rabbits, the author obtained the following results.

1. In case of the stimulation of the sympathetic zone, Qo_2 of the iridociliary body showed 30% decrease in 2 hours after the stimulation and recovered in 6 hours or so. No remarkable change was shown in $Q_M^{O_2}$, but $Q_M^{N_2}$ showed about 20% decrease in 1 or 2 hours after the stimulation.

2. In case of the stimulation of the parasympathetic zone, Qo_2 of the iridociliary body showed 8% decrease in one hour after the stimulation. No remarkable change was shown in $Q_M^{O_2}$ and $Q_M^{N_2}$.

3. By the stimulation of the sympathetic zone, the total nitrogen content in the aqueous humor showed a 2.2 times normal value in one hour and almost recovered in 6 hours.

4. By the stimulation of the sympathetic zone, the protein nitrogen content became 7.4 times greater than the normal value in 2 hours and in 6 hours 1.6 times greater.

5. By the stimulation of the parasympathetic zone, the protein nitrogen content was 1.5 times greater than the normal value in 2 hours and 1.3 times in 6 hours. On the other hand the non protein nitrogen showed a slight decrease.

6. Histologically, hyperemia and edema were shown in the iridociliary body by the stimulation of the sympathetic zone.

7. On the other hand the stimulation of the parasympathetic zone caused no remarkable change in the iridociliary body, histologically.

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ALLERGIC REACTIONS OF SKIN AND CONJUNCTIVA WITH POLLEN IN VERNAL CATARRH

Masami OGUCHI
Eye Clinic of Nippon Medical College

Preface

Today we have various theories of the aetiology of vernal catarrh. Principal ones are:

1) Vogotonia.

2) What is considered as constitutional, especially in the relation to the endocrine glands.

3) Allergic disease of the conjunctiva.

The most popularly accepted theory of these three is that of the allergic disease of the conjunctiva, and the pollen-sensitization-theory as its antigen, is one of the most important. Recently Naiki and Oguchi, the reporter, have advocated the existence of an acute vernal catarrh, and asserted, that it is the primary symptom of ordinary vernal catarrh. On this subject, we have a detailed report prepared by Ueoka and Ito. Furthermore, Naiki and this reporter, observing the skin reaction against pollen, pointed out that it showed an allergic nature. Afterwards, this reporter made a series of experiments on both patients and non-patients of vernal catarrh as to the reactions of skin and eye. These experiments have brought out a conclusion to believe that an acute vernal catarrh was experimentally developed. The following report is hereby made based on these experiments.

Method of Experiments

The pollen employed by this reporter were those of anemophilous flowers including Pinus thunbergi, Salix gracilistyla and Alopeculus aequalis, and of entomophilous flowers including Camelia japonica and Chrysanthemum cinerariaetolium. The pollen were sufficiently pulverized after collected and were conserved at all times in a dry oven. In the experiments on skin reactions, the reporter employed the method of scarifying the skin of either upper fore arms. As solvent, the reporter used 0.2 per cent solution of caustic soda. Judgements were based on the findings 20 minutes after the beginning of the process. In the meantime, utmost care was paid, lest we should overlook the drying

of the solvent, and the solution was added as the situation demanded. The following standard was provided in order to mark the positive reaction of the skin.

Absolutely negative reaction (−)

The red circle showing the positive reaction
- below 10 mm. (+)
- below 20 mm. (++)
- above 20 mm. (+++)

Occasionally it came to the attention of the reporter, that a flat exanthem or induration in the center of the circle showed itself, but this matter will not be taken up on this occasion.

To test the reaction on the conjunctiva, the pollen were dropped in one eye and powdered milk into another so as to make a comparative study of these two cases. The eyes were kept closed for 20 minutes. After that the conjunctiva was washed, the effects were observed and the secreta examined (mainly employing the Giemsa staining). The positivity of eye reaction was defined according to the following standard.

The case of a non-patient who showed an allergic reaction to pollen. If hyperaemia conjunctiva or edema and / or an eosinophile cell was noticed, such was to be defined as positive. If the congestion or edema was slight or absent and / or an eosinophile cell was not noticed in the secreta, negative.

The case of vernal catarrh patients. This test was carried out in December as a wintry experiment.

In each case, pollen found to be allergic in skin reaction experiment were used. Those whose eye symptoms showed an aggravation and / or if those who had no eosinophile cell in the secreta before the dropping of pollen, developed an eosinophile cell they were termed "positive". Otherwise, they were "negative".

Results

A) Reaction of skin and eye in case of nonpatients is shown in Table 1.

Table 1. Skin and Eye Reaction in Normal Eye

No.	Name.	Age.	Gender	Skin reaction	Eye-reaction	
1	Sekiguti	18	♀	Alo(+++) Pin(+)	Alo(+)	on 3rd day Hyperemia E(+)
2	Fukushima	18	♀	Alo(++) Pin(+)	Alo(+)	on 3rd day Hyperemia E(+)
3	Niiyama	41	♀	Sali(+++)	Sali(+)	on 2nd day Hyperemia E(+)
4	Hoshi	19	♀	Pin(++) Sali(+)	Pin(−)	
5	Yamamoto	18	♀	Pin(++) Alo(+)	Pin(−)	
6	Ono	21	♀	Alo(++)	Alo(−)	
7	Hasumi	22	♀	Pin(+) Sali(+)	Pin(−)	
8	Morishige	21	♀	Alo(+++)	Alo(−)	

: Alo=Alobeculus aequalis: Sali=Salix gracilistyla: Pin=Pinus thunbergi
: E=eosinophile Cell in Secreta

The skin reaction experiments on dozens of nonpatients showed a considerable number having been transformed into the positive. Eight persons wers experimented on as to the reaction on the conjunctiva. In skin reaction, five were positive (Alopeculus aequalis), five (Pinus thunbergi), and three (Salix gracilistyla). These also include four positives affected by more than two kinds of pollen.

In conjunctiva reaction, the reporter used pollen of higher possibilities of skin reactions. The positive reaction was noticed in three among the eight viz., No. 1 Sekiguchi, No. 2 Fukushima and No. 3 Niiyama. No. 1 and No. 2 showed that hyperaemia conjunctivae and eosinophile cells in the secreta continued for three days. The fourth day hyperaemia conjunctivae resolved along with the disappearance of eosinophile cells in the secreta. No. 3 showed that hyperaemia conjunctivae and eosinophile cell in the secreta continued for two days. The various symptoms disappeared on the third day. Meanwhile, these three had discharge at the time of starting up, but lacked the subjective symptoms of pruritus and photophoby. A very small proportion of eosinophile cells was noticed in the secreta.

B) Eye reaction test applied to vernal catarrh patients in winter-time produced results as shown in Table 2.

Six patients mentioned here except No. 6 Numata had been diagnosed as vernal catarrh between spring and summer. Each patient had been convalescing or completely recovered by the time of the test. The result of the test was as follows:

Case 1. Ito, Age; 12♂. Ist day Diagnosis. Ocular type. Skin reaction: Pinus thunbergi positive. December findings neither subjective nor objective. No E-cells in secreta. Pollen powder of Pinus thunbergi into right eye, milk powder into left. After 20 min. eye wash. Normal in left eye. Conj. bulbi of reight eye indicates hyperemia of hight degree and edema found. E-cells found in secreta on Ist day. 2nd day edema disappeared, its hyperemia and E-cells in the secreta decreased a little. 5th day, all symptoms disappeared. Decision of eye-reaction, positive.

Case 2. Yoshida, Age;13♂. Ist day Diagnosis: Palpebro-ocular type. Skin reaction: Alopeculus aequalis (++), Pinus thunbergi (+). Views in December, Stonewall like proliferation of papilla dicernible on palpebral conj. and no hypermia. Perilimbal conj. slightly colored. Neither photophoby nor feeling of itch. E-cells of middle grade discernible in secreta. Pollen-powder of Alopeculus aequalis into right eye and milk powder into left. After 20 min. eye-wash. No excerbation of left eye symptoms. Bulbar conjunctival hyperemia of right eye advances highly and edema appears. Coloration of limbus conj. increases and papllary proliferation beccomes considerable. E-cells in secreta highly increase. 2nd day conjunctival hyperemia and edema decreases a little. 3rd day same condition with some photophoby. Same condition continues till 9th day. Decision of eye-reaction positive.

Case 3. Kameda, Age; 31♂. Ist day Diagnosis: acute vernal catarrh. Skin reaction; Pinus thunbergi (++). Views in December neither subjective nor objective. Pollen powder

Table 2. Eye Reaction in Vernal Catarrh Patients in Winter

No.	Name	age	gender	Passed anamnesis	Status presens		Skin reaction	Eye reaction						Process		Decision
								Kind of pollen powder	Pollen powder into right eye		Milk powder into left eye				
					Symptom	E			Symptom	E	Symptom	E	Symptom	E	
1	Ito	12	♂	bulbar type	(−)	(−)	Pin (+)	Pin	hyperemia (++) edema (++)	(−)	(−)	(−)	2nd day (+) 3rd day (−) 5th day (−)	+++ + −	(+)
2	Yoshida	13	♂	bulbar type	(±)	(++)	Alo (++) Pin (+)	Alo	hyperemia (++) edema (+++)	(+++)	(±)	(++)	2nd day (++) 3rd day (++) 9th day (++)	(++) (++) (++)	)+)
3	Kameda	8	♂	acute	(−)	(−)	Pin (++)	Pin	hyperemia (+) edema (−)	(+)	(−)	(−)	2nd day (−)	(−)	(±)
4	Tsunoda	8	♂	acute	(−)	(−)	Pin (+)	Pin	hyperemia (+) edema (+)	(−)	(−)	(−)	2nd day (−)	(−)	(±)
5	Nakamura	18	♂	palpebrobulbar type	(±)	(+)	Sali (++)	Sali	hyperemia (++) edema (−)	(+)	(−)	(+)	10th day (+)	(+)	(±)
6	Numata	16	♂	acute (autumnal)	(+)	(+)	(−)	Pin	hyperemia (+) edema (−)	(+)	(+)	(+)	10th day (+)	(+)	(−)

: Acute=acute vernal cattarrh : E=eosinophile cell : Pin=Pinus thunbergi : Sali=Salix gracilista
: Alo=Alopeculus aequalis

of Pinus Thunbergi into right eye, powder milk into left. After 20 min. eye wash. Normal in left eye. In right eye, conjunctival hyperemia, but no edema. In the secreta, a small number of E-cells. 2nd day, conjunctival hyperemia disappears and no E-cells in discharge. Decision of eye reaction positive.

Case 4. Tsunoda, Age; 8♂. Ist day Diagnosis: acute vernal catarrh. Skin-reaction: Pinus Thunbergi (+). December view neither subjective nor objective. Polln powder of Pinus thunbergi into right eye, milk powder into left. After 20 min. eye-wash. Normal in left eye. Conjunctival hyperemia and edema slight. E-cells in secreta negative. 2nd day neither hyperemia nor edema. No E-cells, either. Decision false-positive. (E-cells negative)

Case 5. Nakamura, Age; 18♂. Ist day Diagnosis: Palpebro-ocular type. December findings: slight coloration of limbus conjunctiva. Skin reaction: Salix gracilistyla (卄). Palpebral conjunctiva opalescence. Pollen powder of Salix gracilistyla into right eye, milk powder into left. After 20 min. eye wash. Normal in left eye. Hyperemia of right bulbar conjunctiva increases slightly. but no edema. Number of E-cells in secreta same as before treatment. Hyperemia of right conjunctiva remains until 10th day. Decision of eye reaction false-positive.

Case 6 Numata. Age 16♂. Ist day diagnosis: acute vernal catarrh (autumn). First day of this case is 10th December and diagnosed to be autumnal acute vernal catarrh. The symptoms similar to acute vernal catarrh above mentioned. All skin reactions negative. Pollen powder of Pinus thunbergi put into right eye in experimental meaning, but exceration of symptoms none. Decision of ocular reaction negative.

Summarization and Consideration

A) On the reaction of skin and conjunctiva in case of nonpatients.

Dozens of non-ailing youths have been tested with various kinds of pollen. Many showed allergic reactions. In some cases, only one kind of cutan-anaphylactic pollen caused the reaction. In Others, more than two did. I picked up eight from these youths and carried out eye experiments with cutan-anaphylactic pollen. As a result, positive reaction was noticed in three. The congestion of the lower half of bulbar conjunctiva was the main effect. This part was where the pollen were contracted. Hyperaemia conjunctivae in other parts was very slight. Neither much of edema in the conjunctiva was discernible.

The reporter noticed eosinophile cells in the secreta in the post-experiment stage, but no one had the subjective symptom sud as pruritus anch photophoby. In the case of example No. 1 and No. 2, hyperaemia conjunctivae and eosinophile cells were noticed until the third day of the experiment. The same was noticed until the second day in the case of example No. 3. This conjunctiva reaction is believed to be an allergic reaction. And as this shows the same symptom as a acute vernal catarrh, it is deemed appropriate to conclude that it is an experimental acute vernal catarrh. The reason why this disease

was cured in a short time is quite simple and there is no room for doubt; as the pollen were applied only once and in case of acute vernal catarrh, many recover in about ten days. But one has a very good reason to entertain a doubt as to the name of experimental acute vernal catarrh. With this in view, therefore, this question will now be dealt with.

1) Peshkin applied powdered pollen to an eye of an asthma-patient. After a few minutes, he noticed hyperaemia conjunctivae and edema. 2) Mr. Kitsukawa, prosecuting his studies of hair-dye-conjunctivitis and pepton anaphylaxis noticed experimentally and clinically a symptom that seemed to be acute vernal catarrh. 3) Mr. Kunitomo, in his study of allergic conjunctivitis, proved, anatomically and clinically, a resemblance between acute vernal catarrh and hair-dye-cunjunctivitis. In these three cases, the symptoms might be similar or identical to those shown in the experimental acute vernal catarrh. Now, why the positive reaction of conjunctiva with pollen is to be called the "Experimental Acute Vernal Catrrh."

Pepton-anaphylaxis and hair-dye-conjunctivitis will have the same primary symptoms as acute vernal catarrh. But the first three are from seasonal characteristics and unless deliberate experimental methods are employed, it will never be chronic. On the contrary, the latter is pregnant with the possibilities of seasonal characteristics and in some cases, it is potentially chronic. Pollen can be the only factor that is linked with season. Even if the primary symptoms are identical, it is natural that the type of the disease is different and the progress not the same. For the reasons cited above, the reporter calls Peshkin's case and this experiment, experimental vernal catarrh. Hitherto, the difficulties in the study of vernal catarrh lay in the seasonal onset and chronic progress. But the above explanation will lead to an easy resolution of these difficulties.

The reporter has now to confess to his inability to give a satisfactory explanation why five persons with positive skin reactions showed negative reactions of conjunctiva. But, generally speaking, the frequency of positive allergic reaction was about the same, and this gives some chance to explain the matter.

B) On the conjunctiva reaction of vernal catarrh patients in winter-time.

The opponents of the pollen-sensitization-theory have been asserting, that occasional onsets of vernal catarrh in the depth of winter have been recorded. But as the floral calendar of Messrs. Misawa and Hisauchi shows, even in the depth of winter some anemophilous flowers, though small in number, bloom. There are many cases in which vernal catarrh developed in the autumn chill. This is why this reporter carried out the experiments of the patients who had the onset of vernal catarrh in late spring or early summer for the purpose of studying the relapse. The result of this experiment is as already related. Three patients out of six suffered a relapse and the symptoms were similar to those attached in spring and summer. The conjunctiva reaction of two patients was false-positive while that of one did not show any aggravation. (This patient had the onset in autumn and whose skin reaction was negative. This was a test experiment.)

The duration of the symptoms of cunjunctiva in the case of three patients who appa-

rently had a relapse was from two to nine days and the shortness of this duration is similar to the case of the experimental acute vernal catarrh. This can be explained by the fact that the application of pollen was just once.

Lehrfeld also noticed an example of an experimental relapse. The continuation of the symptoms of conjunctiva is also short in this case. The experiment by the use of pollen in winter afforded a more favorable basis for the pollen-sensitization-theory. There is much argument as to the reliability of reactions of skin and conjunctiva and the difference between the acuteness of these two, etc. The disagreement between skin and coniunctiva reaction must be an object of future study.

CONCLUSION

1. Pollen were dropped in the eyes of eight non-patients and symptoms of an acute vernal catarrh in three were noticed. This was named the "*Experimental Acute Vernal Catarrh.*"
2. Pollen allergic to skin were dropped in the eyes of six vernal catarrh patients in winter-time and a relapse in three patients was perceived.
3. The pollen-sensitization-theory is considered to be most dominant.

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BIOCHEMICAL STUDIES ON THE CHANGES OF THE CORNEA AND AQUEOUS HUMOR AFTER DEATH

Tuneyuki AOYAGI

Department of Ophthalmology, School of Medicine, University of Tottori

It is obvious that the success of corneal transplantation is closely related to the freshness of the donor eyeballs which must be enucleated and preserved after death as soon as possible. The exstirpation of the eyeball after death is largely restricted by the national habits and religious influences and must be delayed for some time to get permission of the relatives.

The purpose of this experiment is to clarify the biochemical changes of the cornea and aqueous humor after death and to determine the best method for preservation of the donor eyeball by the biochemical results.

Materials and Methods

Enucleated bovine eyeballs were employed as experimental materials. The reductic ascorbic acid and glutathione, which were importantly connected with the oxydoreductic reaction, and the weight of the cornea, which was related to the water metabolism, were surveyed under various conditions and preservation methods.

The quantitative determination of reductic ascorbic acid was examined by the instillation of 2.6-dichlorphenolindophenol by Fujita and the Teruuchi and Shimada modified method using 2.4-dinitrophenolhydrazin (DNP). The measurement of reductic glutathione was employed the iodine instillation method originated by Fujita and Iwatake.

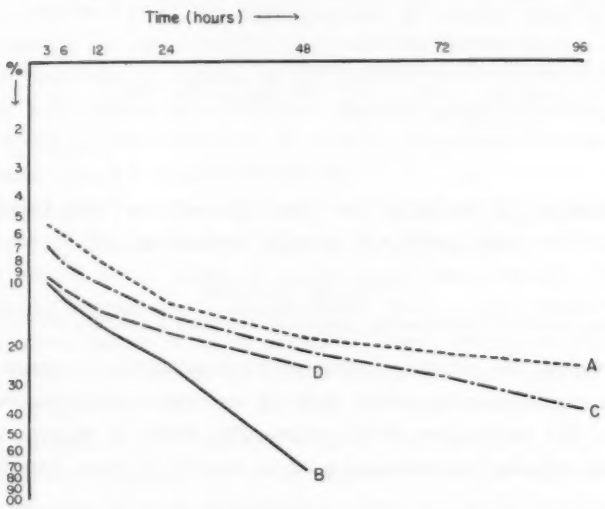
Time trend diagrams were drawn by the logarithmic results of decreasing or increasing percents.

Results

1. Cases of preservation under room temperature in winter and summer

Decreasing rate of reductic ascorbic acid and glutathione of aqueous humor. In summer (27°C~30°C), reductic ascorbic acid decreased severely, 7.45% in an hour, 10.32 in three, 12.42 in six and then gradually 16.21% in twelve, 24.39 in twenty-four, 36.36% in thirty-six and 75.99 in fory-eight hours. In forty-eight hours it decreased to three quarters as shown by the B curve in figure 1.

Fig. 1 Time trend diagram of semilogarithmic scale of decreasing rates of reductic ascorbic acid and glutathione on aqueous humor at room temperature.



A……Reductic ascorbic acid in winter (1.2°C-4.5°C)
B……Reductic ascorbic acid in summer (27°C-30°C)
C……Reductic glutathione in winter
D……Reductic glutathione in summer

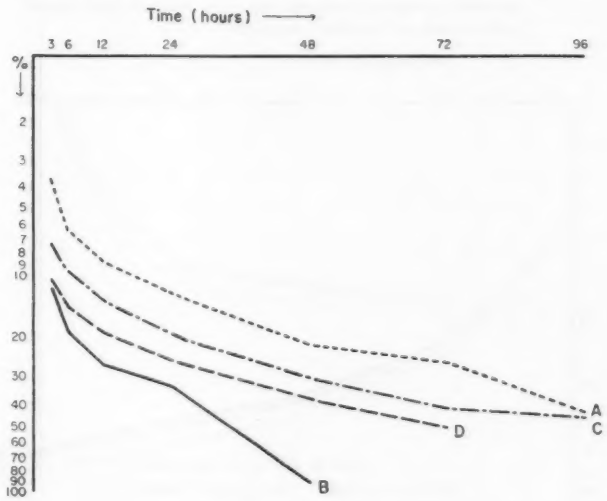
In winter (1.2°C~4.5°C), it decreased very slowly and gradually as shown by the A curve. Even if at ninety-six hours it lost only 25.36% and at forty-eight 18.7% which was almost equal to that of eighteen hours in summer.

Upon the reductic glutathione, it decreased promptly in three hours 10.75% and then gradually, that is, 14.58 in six, 18.92 in twelve, 25.68 in twenty-four and 38.17% in forty-eight hours, in summer. In three days, the aqueous humor was almost so lost that it could not be measurable. Compared with reductic ascorbic acid it decreased very slowly and became more indistincts as shown by the C curve in figure 1. Even after 2 days it remained yet at 60 percent.

The reductic glutathione of the aqueous humor decreased slower and became more indistinct, in winter, that is, in three hours this decreasing rate was only 6.93, in six 8.41, in twelve 9.89, in twenty-four 14.75, in forty-eight 21.79, in seventy-two 28.22 and in ninety-six hours 40.12 which was slightly higher than that of forty-eight hours in summer.

Dereasing rate of reductic ascorbic acid and glutathione of the cornea. Upon reductic ascorbic acid, it decreased in summer, 11.96% in three, 18.80 in six, 26.22 in twelve, 33.84 in twenty-four, 93.99 in forty-eight, as shown by the B curve in figure 2. It is very interesting that it decreased earlier than that of the aqueous humor and was almost completely

Fig. 2 Time trend diagram of semilogarithmic scale of decreasing rates of reductic ascorbic acid and glutathione of the cornea at room temperature.



A·····Reductic ascorbic acid in winter
B·····Reductic ascorbic acid in summer
C·····Reductic glutathione in winter
D·····Reductic glutathione in summer

lost in twenty-four hours.

In winter, it decreased much slower and gradually than that of summer, that is 3.68% in three, 6.47 in six, 8.92 in twelve, 12.78 in twenty-four, 21.98 in forty-eight, 26.35 in seventy-two and 44.36 in ninety-six hours as shown by the A curve. It ranged from only one third to one fifth of that of summer. Even after five days, the cornea contained more ascorbic acid than one half of the normal stage.

From the stand point of reductic glutathione it decreased promptly in three hours 10.75% and then gradually that is, 14.58 in six, 18.92 in twelve, 25.68 in twenty-four, 38.17 in forty-eight, 52.35 in seventy-two hours in summer as shown by the D curve.

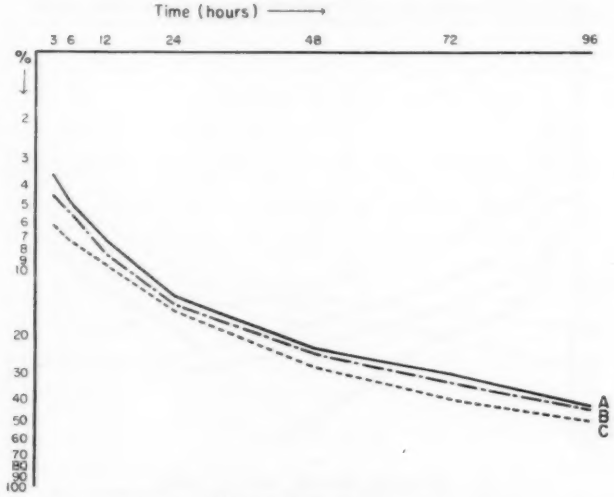
In winter, it decreased, however, much slower than that of summer, that is, 7.39 in three hours, 9.96 in six, 13.45 in twenty-four, 30.58 in forty-eight, 42.19 in seventy-two and 47.52 in ninety-six hours, as shown by the C curve in figure 2.

The decreasing rate of glutathione of the cornea was smaller than that of ascorbic acid and even after four days the cornea contained more glutathione than one half of that of the normal stage.

2. Cases of preservation into various solutions

The enucleated bovine eyeball was put into a bottle containing bovine serum or Ringer's solution and kept in a refrigerator with a temperature of 1-4 C. When the physiologic saline solution was used the eyeball was put on the gauze in the bottle soaked with the solution, which was kept afterwards in a refrigerator.

Fig. 3 Time trend diagram of the semilogarithmic scale of the decreasing rate of reductic ascorbic acid of the cornea preservation of various solutions.



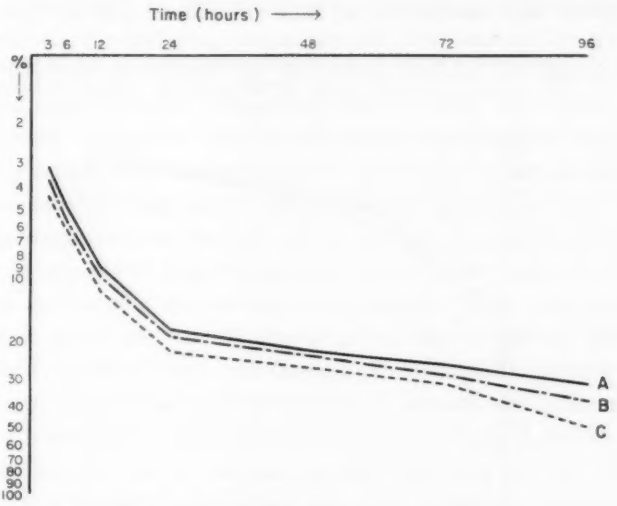
A·····Preservation in the serum
B·····Preservation in the Ringer's solution
C·····Preservation in the physiologic saline solution

Decreasing rate of reductic ascorbic acid of the cornea. When the eyeball was kept in the serum, the decreasing rate of reductic ascorbic acid was 7.83% in three, 9.09 in six, 11.59 in twelve, 17.76 in twenty-four, 27.08 in forty-eight, 34.76 in seventy-two and 46.27 in ninety-six hours as seen the A curve; in the Ringer's solution, 10.84 in three, 11.87 in six, 14.64 in twelve, 19.86 in twentyfour, 31.51 in forty-eight, 40.57 in seventy-two, 50.25 in ninety-six hours as shown by the B curve; and at the physiologic saline solution, 12.96 in three, 14.90 in six, 18.09 in twelve, 23.65 in twenty-four, 35.29 in forty-eight, 43.93 in seventy-two and 51.81 in ninety-six hours as shown by the C curve in figure 3.

From this result it revealed that the value of the serum was the smallest and best method for preservation, next in the Ringer's solution and the last in saline solution. All of them decreased gradually and almost in equal tempo. Even after four days, it decreased only below fifty percent.

Reductic ascorbic acid of the aqueous humor decreased in the same order than that of the cornea and its decreasing rate was almost one half of that of the cornea. Reductic ascorbic acid of the cornea decreased more rapidly than that of the aqueous humor.

Fig. 4 Time trend diagram of the semilogarithmic scale of the decreasing rate of reductic glutathione of the cornea under preservation of various solutions.



A……Preservation in the serum
B……Preservation in the Ringer's solution
C……Preservation in the physiologic saline solution

Decreasing rate of reductic glutathione of the cornea. At the preservation in the serum, reductic glutathione of the cornea decreased gradually, that is 8.12% in three, 12.43 in six, 19.69 in twelve, 27.16 in twenty-four, 36.88 in forty-eight, 39.61 in seventy-two and 48.48

in ninety-six hours, as shown by the A curve; in Ringer's solution 8.64 in three, 13.82 in six, 21.79 in twelve, 30.66 in twenty-four, 40.60 in forty-eight, 48.68 in seventy-two and 54.96 in ninety-six hours, as shown by the B curve; in the physiologic saline solution 9.21 in three, 16.79 in the physiologic saline solution 9.21 in three, 16.79 in six, 25.96 in twelve, 34.46 in twenty-four, 45.25 in forty-eight, 51.73 in seventy-two and 69.20 in ninety-six hours, as shown by the C curve in figure 4.

All of them decreased slowly and gradually showing an almost equal decreasing rate, between them, the decreasing rate was, however, the greatest at the saline solution, then came Ringer's solution and the smallest at the serum.

Even after four days, reductic glutathione of the cornea remained over fifty percent.

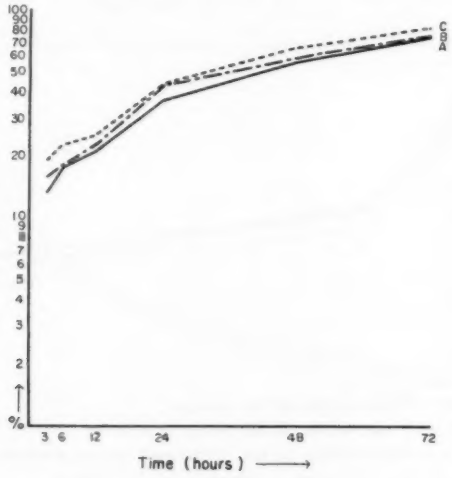
The decreasing rates of reductic glutathione of the aqueous humor were far smaller than those of the cornea. At first, the difference between them was almost an one to two ratio, becoming three to five in four days.

It is very interesting that these rates were almost the same at the reductic ascorbic acid.

The increasing rate of corneal weights under preservation in various solutions. When the solution for preservation was that of the serum, the corneal weight gained gradually and steadily by the absorbing fluid from the serum and aqueous humor its increasing rates were 13.1% in three, 17.4 in six, 20.9 in twelve, 36.4 in twenty-four, 55.9 in forty-eight and 73.0 in seventy-two hours, as shown by the A curve in figure 5.

The same effects were observed at the Ringer's solution, that is, 15.6% in three, 17.9

Fig. 5 Time trend diagram of the semilogarthmic scale of the increasing rate of corneal weights under preservation in various solutions.



A……Preservation in the serum
B……Preservation in the Ringer's solution
C……Preservation in the physiologic saline solution

in six, 22.5 in twelve, 42.7 in twenty-four, 58.3 in forty-eight and 75.7 in seventy-two hours which were slightly higher than those of the serum solution as shown by the B curve.

At the physiologic saline solution, the increasing rate was the highest, that is 18.8% in three, 22.4 in six, 25.1 in twelve, 43.9 in twenty-four, 65.7 in forty-eight and 81.2 in seventy-two hours, as shown by the C curve in figure 5.

From these results the serum is the best solution and the saline solution the worst one for preservation.

When the eyeball was kept under naked condition at room temperature the weight of the cornea also increased gradually becoming over one half in seventy two hours in winter as in summer. This increasing rate of the cornea under naked condition was far lower than that under preservation in various solutions, even if in the serum. This was induced by the infiltration of aqueous humor into the cornea diminishing the water content of the aqueous humor.

Discussion

The metabolism of the cornea after death was reported by Duane, Robbie & Leinfelder, Kora, Shimoyama, Nakamura et al. and Kasahara. Among them, Kasahara experimented on the difference of the remaining vital rate induced by the changes of solutions for preservation which were used, e.g, serum, Ringer's, physiologic saline solution and aqueous humor. His result was the following ; serum was the best solution for preservation and the remaining vital rate the highest, the next better Ringer's and physiologic saline solution and the worst aqueous humor. This result was the same with mine. According to Miyaura's experiment of ascorbic acid of the eyeball, especially the cornea and aqueous humor decreased gradually and became loosing 42 percent at the cornea and 52 percent at the aqueous humor in seven days which was a smaller percentage than in my results. Owing to my experiment reductic ascorbic acid of the cornea was almost completely (94%) lost in two days in summer at room temperature but in winter only at 44 percent. The decreasing rate of the reductic ascorbic acid of the aqueous humor was smaller than that of the cornea, that is, it was only 76 percent in two days in summer and 25 percent in winter. According to the time trend, the diagram of the reductic ascorbic acid, the decreasing rate showed a linear curve in three hours in summer and in six hours in winter and then the very much slower and accelareting curve, as shown in figures 1 and 2.

When the eyeball was put in various solutions and kept in the refrigerator, the reductic ascorbic acid decreased more slowly than that of winter. There was almost no difference of the decreasing rate between the preserving solutions in twenty-four hours, after that the decreasing rate of the serum was the lowest, that of the Ringer's solution followed and of the saline solution the highest, that is, the serum was the best solution for preservation of the eyeball as shown in figure 3.

There was almost no experimental report of the decreasing rate of reductic glutatione

of the cornea and aqueous humor after enucleation. They decreased gradually at room temperature, as time went on. The decreasing rate of the cornea was greater than that of the aqueous humor, as shown in figures 1 and 2.

When the eyeball was preserved in the varions the decreasing rate of the cornea was slightly higher than that of winter and lower of summer. Between the preserved solutions the decreasing rate of the serum was the lowest and that of saline solution the highest, as shown in figure 4.

The increasing rate of corneal weight under preservation in various solutions was shown in figure 5. At that experiment the cornea absorbed the fluid from the preserving solutions as well as of the aqueous humor. Between them, the increasing rate of the corneal weight in serum was the lowest and that of saline solution the highest. The increasing rate of the preserving in the naked state at room temperature was far lower than that one preserved in serum.

Summary

1. Reductic ascorbic acid and glutathione in the cornea and aqueous humor decreased gradually at room temperature. The decreasing rate in summer was higher than that of winter.

2. When the eyeball was preserved in various solutions, reductic ascorbic acid and glutathione decreased slowly showing an almost equal decreasing rate, which was, however, the highest at the saline solution and the lowest at the serum.

3. When the eyeball was preserved in various solutions the weight of the cornea gained gradually by the absorbing fluid from the aqueous humor as well as the solution for preservation.

4. From these biochemical results the best method of preservation was to keep the eyeball in low temperature and at moderate humidity.

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TONOGRAPHIC STUDIES ON HUMAN EYES
Report 4 STUDIES ON THE SQUINT EYES

Keikichi IKUTA
Department of Ophthalmology, Okayama University Medical School

Introduction

When the problem of ocular tension is discussed, the impediment in the outflow of aqueous humor is an important factor for the increase of the ocular tension, whether retention theory is acknowledged or not. Yet there are many different views on the mechanism of the regulation of the ocular tension, and no definite conclusion has been reached. To know if the mechanism is simply anatomic or physical, or physiological and psyconeurotically physiological, it is necessary to make it clear how the outflow is affected under various physiological and pathological conditions. The author compared the facility of the outflow of the aqueous humor of the human eye in abnormal cases by tonography with one in the normal case. In case of the normal eye, the facility of aqueous outflow increases by accomodation, and in case of the severe refractive anomaly the facility of aqueous outflow of the eye of hyperopia inclines to be less than that of the eye of myopia. It was considered, that this was caused by the more frequent accomodation in case of hyperopia and this compensates for the usual small amount of outflow. It was concluded that the function of the intrinsic ocular muscles has a great influence on the outflow besides the resistance of outflow and anatomic and mechanical conditions, when the morbidity of glaucoma is put into consideration. It was further concluded that the decreased C of the simple glaucoma is much improved by filtration operation. But as there was some impediment in outflow in the latter 2 minutes of the 4 minutes' tonogrophy, it was imagined that there is a limit, through the outflow of aqueous humor is accentuated by the enlargenment of the morphological outlet, and that there is functional regulating factors of the superior centre. Yet these researches were about the influence of the mechanism inside the eyeball, given to the outflow. But on the other hand, it is reported that the action of the extrinsic ocular muscles causes the increase of the intraocular tension after the advancement operation of the squint, or on the contrary the decrease of the ocular tension by administration of curare was observed.

Since it was imagined that the tonicity of the extrinsic ocular muscles would also affect the intraocular tension and the facility of the outflow, a research was was made about the change of C of both the squinting eye and of the corrected eye using tonography.

Materials

In this experiment, male and female patients of concomitant convergent and divergent squint under 30 years of age, who have normal ocular movement, and have no other diseases were used. First the author tried to measure C of the normal eye turning

sideward, but gave this up because the cornea was too much at one side of the palpebral fissure and it was hard to place the plunger in the right position.

Method

Müller's electronic tonometer was used. The room used was a room facing northward so that it may be affected least by outside circumstances. The illumination was 50 lux. The patient was laid on his back on a bed and a small lamp which could be moved in any direction was fixed 4 metres aport from the patient's eye. The lamp was fixed by the patient with his master eye so that the visual line of the axis of the squint eye might be fixed vertical. Then tonography was done on it in that position to measure out C. Then tonography was again done on the eye of the patient whose eye was corrected by recession and who has no diplopia and is entirely free from irritation and then comparison was made between, before, and after the operation.

Results

As the table shows, the facility of the aqueous outflow increases more after the operation than before the operation, and the intraocular tension is higher before the operation than after. So it is possible to acknowledge the significance of the difference between before, and after the operation, when the possibility of there being 5% of danger is admitted.

Table 1. Comparison of pre Operative C and Po with Post Operative

case	pre operative			post operative	
No.	sort	C	Po	C	Po
1	conv.	0.491	21.12	0.603	15.14
2	〃	0.178	21.47	0.286	15.66
3	〃	0.444	24.08	0.634	17.30
4	div.	0.180	21.82	0.308	18.50
5	conv.	0.390	20.77	0.417	18.80
6	〃	0.218	12.80	0.216	10.58
mean	——	0.317	20.34	0.410	16.14
range in 5% danger	——	±0.148	±4.07	±0.174	±3.16

Each deviation is significant in 5% danger

Discussion

1) As a possible factor which affects the outflow and the intraocular tension, we can think of the change in the form of the eye ball and the increase of the intraocular tension due to the tonicity of the extrinsic ocular muscles.

In normal eyes, each extrinsic ocular muscle has tonicity and is kept in equilibrated position even in the physiologically static state free from the impulse caused by fusion.

This fact will be proved by the following results:

Hoffmann et al. observed, that a 60~100 vibrating action current of 60~100 cycles is in the extrinsic ocular muscles. And Pulfrich also found, that extremely fine action current could be detected even at the time of rest. When V. Hippel and. Grünhagen measured the intraocular tension of the animal, they observed the decrease of the intraocular tension, as the extrinsic ocular muscles are paralized, when curare was used as sedative. Duke-Elder obtained the same result using a dog, and Hugson's result was the same with the human being. Kirby admitted the decrease of the ocular tension at the time of the cataract operation using curare, and H.Hoffmann and. H.Holzer measured out the decrease of the ocular tension using decametonium and succinyl bischolinester. On the other hand, as Adler pointed out, Sherrington's law concerning the eye ball at the time of its movement does not mean, that the antagonistic muscles cannot contract at the same moment, but it means that the rate of the contraction is not the same on each antagonistic muscle at one same moment. But as the extrinsic ocular muscles act according to Sheirington's law of mutual innervation, there are still some questions about the increase of the pressure given to the eye ball by the extrinsic ocular muscles, when the eye ball makes a movement. Yet Donders says that the contraction of the internal rectus muscles caused by convergence and the strain of the external rectus muscles gives pressure to the eye ball and brings stasis to it causing myopia. And Mannhar and Wakizaka also support the idea of the pressure given to the eye ball by extrinsic ocular muscles; and Phillip says that the myopia is caused by the pressure on the eye ball given by the superior oblique muscle. These are not exactly the studies about the circulation of aqueous humor, but they are enough to show the possibility of the strain of the extrinsic ocular muscles to give pressure to the eye ball. According to the present experiment, the intraocular tension when the squint eye ball is forced to turn to the front is greater than that of the eye ball facing to the front without tonicity of the ocular muscles after tenotomy. It is possible to think, that one cause of this is the pressure given by the extrinsic ocular muscles.

2) The pressure on the eye ball given by the extrinsic ocular muscles not only increases the intraocular tension but also has the possibility of hindering the flexibility of the sclera when the tonometer is placed on the cornea. Schiefferdecker and Kato admitted, that ocular muscles consist of lots of elastic fibers. As it is possible to consider this group of the ocular muscles as one elastic body, the eye ball is pulled backward by the tonicity of four rectus muscles, and is also pulled forward by two oblique muscles, when the eye ball is in a static state; and so, when the force given to the sclera by the ocular muscles increases, the coefficient of rigidity of the sclera appears to be greater, and naturally the facility of aqueous outflow is likely to be calculated smaller than actual, if the standard coefficient of rigidity is used in calculation. Also Po will be estimated greater. So it is imagined that the actual difference of ocular tension between before and after the operation is actually smaller, than the one shown in the table.

3) Since each opening of these four vortex veins is located 6 mm. backward from

the equator, it never happens, that the openings are pressed even if the eye ball is moved in the maximum degree taking the anatomical and physical point of insertion including the tangential arc into consideration. On the other hand, the upper opening of the lateral vortex vein is rignt under the point of insertion of the superior oblique muscle and is always covered with the superior oblique muscle; and the lateral lower opening is near the point of insertion of the inferior oblique muscle and is likely to be under the direct influence of the action of the muscle. This fact was also pointed out by Fuchs and Weichelbaum. And since both superior and inferior oblique muscles take parts in the abduction, Arlt also afirmed the fact that the lateral rectus muscle and the inferior oblique muscle give pressure to the vortex veins bringing stasis into the eye and promote myopia. As a possible factor concerning the values obtained by tonography, we can imagine that the resistance of the outflow will be increased by the compression on the outlet of the aqueous humor iucluding the chamber angle, by the increase of the volume of the iris and the ciliary body caused by stasis.

The congestion of the iris and the ciliary body brings on the increase of the intraocular pressure due to the increase of the volume of the iris and the ciliary body itself as well as of the choroid, and the increase of the formation of the aqueous humor. Kishimoto and many other authors think that the aqueous humor flows to the vortex veins, besides Schlemm's canal and aqueous veins, through part of the ciliary body and choroidal vessels and it finally goes to the vortex veins, first absorbed into the surface of iris and then passing through the vessels of the ciliary body and choroid. But if these facts are true, in the case of strained oblique muscles, there must occur hindrance in the outflow directly caused by the vortex vein. As for the elevation of the ocular pressure due to the stasis inside the eye, many authors after Wessely, think that the reason why the ocular pressure is increased by the pressure on the neck, the ligature of the jugular vein or by the anteflextion of the neck is, that stasis takes place in the eye, and that the stimulation on the cervical sympathetic nerve has also connection with the increase. As for the outflow, Kitajima acknowleged by tonography, the decrease and instability of the facility of outflow, when pressure was given on the neck of a rabbit, and says, that this was caused by the repetition of the accentuation of the ocular pressure due to the stasis inside the eye and by the increase of the pressure of aqueous veins. But Takon says, that there is not much difference in the degree of the influence to the increase of the intraocular pressure caused by the backward outflow.

4) As for the nerve innervating the extrinsic ocular muscles, the sixth cranial nerve innervates the lateral rectus muscle and the fourth cranial nerve innervates the superior oblique muscle, and the others are all innervated by the oculomotor nerve. But, though much discussion has been made by many investigators since Handerson about the relation between parasympathetic nerve and intraocular pressure, they have not come to any agreement in views. Yet it is imagined, that the strain of the autonomic nerve caused by the contraction of extrinsic ocular muscles affects the nervous system reflexively innervating

the regulation of the ocular pressure. Quite different from convergence, as to the influence of the cooperative movement of the extrinsic ocular muscles, to the intrinsic muscles, for instance, in miosis, nothing has been proved.

And there is much to be discussed about the relation between oculomotorius, trochlearis, abducens and Westphal's nucleus. But Professor Akagi acknowledged, that when the rabbit which had had no treatment, the one which had had akinesia and whose four rectus muscles had been cut were tested, the increase of the ocular pressure of the rabbit which had no treatment was greater when its oculomotor nerve was stimulated, but its recovery was faster. Thinking that there would be some other factor for the increase of the ocular pressure besides the tonicity of the ocular muscles, Yamamoto experimented with the rabbit with ligated vortex veins, obtaining the result that the ocular pressure extremely decreases after the temporary increase and the pressure never becomes greater than before, though it increases again, but that if the innervation of the central nerve was removed by cutting off the trigeminal nerve, the second increase far surpasses the first one, So the author thinks, that we cannot deny the influence of the autonomic nerves so far as the pressure of the vortex vein by the extrinsic ocular muscles is admitted.

Conclusion

As for the factors for the hindrance in the outflow of the aqueous humor mentioned above, the increase of the intraocular pressure caused by the pressure of the extrinsic ocular muscles affects C very little but greatly affects F which is directly related to Po as the tonography formula shows. Next, the author wants to mention that the increase of the rigidity of the sclera affects C in smaller degrees in the actual case than what is obtained by calculation, using the standard coefficient. Besides, the various factors, which the author mentioned above concerning the pressure of vortex veins, the act to decrease C. Though which factor influences most is not obvious yet, the decrease of C was clearly shown in the present experiments. It was concluded, that the tonicity of extrinsic ocular muscles not only hinders the outflow of the aqueous humour but also increases the intraocular pressure.

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STUDIES ON KERATITIS SUPERFICIALIS DIFFUSA: THE RIBOFLAVIN METABOLISM IN 30 CASES.

Koichiro EGUCHI

Department of Ophthalmology, School of Medicine, University of Tokyo

Since Miyashita had reported particular investigations on keratitis superficialis diffusa (k.s.d.), the disease attracted much attention among Japanese ophthalmologists. There found many cases in Japan, while, they are rare in Europe and America. In the Text Book of Ophthalmology of Duke-Elder, the author designated it as "a rare disease" and picked up some reports by Japanese investigators.

The chief symptoms of the disease:

a) A fine gray foci on the corneal surface,

b) Maceration on the corneal epithelium and rough surface.

It sometimes appears combined with conjunctivitis or independently in other cases.

Many researches were made in Japan. Takeda obtained a conspicuous curative result by administering Vitamin B_2 to the patients, and insisted that the deficiency of Vitamin B_2 complex would be the cause of the disease. Suda also found a epithelial change in the cornea on the riboflavin deficient rat.

It has been considered that the disease is caused by riboflavin deficiency. While some investigators stated that riboflavin was not effective, but Vitamin B_3 or Vitamin A instead of Vitamin B_2 was effective.

Curative effect of riboflavin is not sufficient, to prove that the deficiency is the real cause of the disease. Therefore, the following experiments were made to study the relationship between the disease and riboflavin.

Method

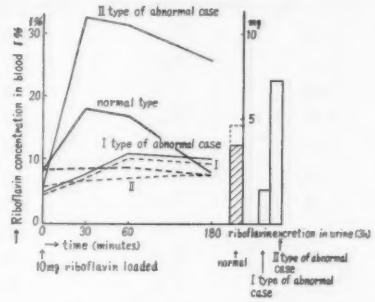
1) Measurement of riboflavin concentration in blood
2) Loading test with riboflavin
3) Observation of symptom for ariboflavinosis
4) Curative effect of riboflavin
5) Changes in 1) and 2), after proper treatments.
6) Schirmer's test for lacrimal secretion.

The lumiflavin-microfluorophotometry (Fujita's method) was used to measure riboflavin contents in blood and in urine, and the bound form of it was determined with benzyl alcohol. For loading test, blood was taken before breakfast. The blood concentration was measured at 30 minutes, 1 hour and 3 hours after subcutaneous injection of flavin mononucleotid (FMN: equivalent to 10 mg. of free type riboflavin). The schema of loading test to normal or abnormal patients are shown in Fig. 1.

In the Ist. type of abnormal case, total riboflavin in blood shows a slight increase after loading, and the bound form also shows a similar curve. Riboflavin in urine decreases.

This indicates the deficiency of riboflavin in the body.

Fig. 1



In the 2nd. type of abnormal case, a great increase of total riboflavin in bood and urine are seen. This means an obstruction in utilization of riboflavin. Discrimination between abnormal Ist. and 2nd. type are possible in 30 minutes after loading.

Results

The average riboflavin concentration in blood of normal persons before breakfast is as follows:

total riboflavin 8.61±1.07γ%, bound form of it 7.77±0.60γ% and ester ratio (bound form/total×100) 91.6%. (Table 1.)

Table 1.

Case No.	Age	Sex	Riboflavin concentration in blood		
			Total R. γ%	Bound form R. γ%	Ester ratio %
1	30	♂	9.0	7.8	87
2	26	♂	6.7	6.6	99
3	28	♂	6.9	7.0	101
4	29	♂	7.7	7.7	100
5	25	♂	10.4	8.1	78
6	25	♀	7.2	7.2	100
7	42	♀	10.0	8.9	88
8	50	♀	10.1	8.7	86
9	35	♀	10.4	8.8	85
10	25	♀	7.5	6.9	92

Experiments were made on 30 patients of k.s.d. who visited our eye clinic from Sept. 1955 to Mar. 1956. As local treatment, only instillation of thiasine solution was done. The data at the first examination are shown in Table 2. Total riboflavin concentration ranges from 1.4% to 16.6% and the average is 7.13±0.32γ%. The bound form concentration ranges from 0.8γ% to 10.9γ% and the average is 5.72±0.29γ%. The ester ratio was 80.6% in average. These values are much lower than the one obtained from normal persons. (Fig. 2) In addition, the more interesting results were obtained when the patients were divided into two groups with normal and deficient lacrimation.

Table 2.

	Case No.	Age	Sex	Riboflavin concentration in blood Total γ%	Bound form γ%	Estel ratio %	Loading test with Riboflavin
normal lacrimation	31	14	♀	1.4	1.4	100	(Ⅰ··· Ⅰ type Ⅱ··· Ⅱ type m···mixed type n···normal)
	32	16	♀	4.1	2.5	61	
	33	25	♀	6.8	5.9	86	
	34	23	♀	7.6	5.3	70	Ⅰ
	35	24	♀	7.0	4.6	66	m
	36	70	♀	5.6	5.3	95	n
	37	24	♀	7.0	7.0	100	
	38	52	♀	5.4	5.9	109	Ⅱ
	39	22	♀	5.3	5.3	100	Ⅰ
	40	15	♂	5.3	1.0	19	Ⅰ
	41	28	♀	5.9	2.6	44	Ⅰ
	42	45	♂	5.5	0.8	15	Ⅰ
	43	19	♀	6.8	5.4	79	Ⅰ
	44	7	♂	6.9	3.5	51	
	45	22	♀	10.1	9.3	92	
	46	20	♀	4.7	4.0	85	Ⅰ
	47			6.7	6.6	99	n
	48	27	♀	10.0	7.3	73	
	49	22	♀	9.8	8.8	90	Ⅰ
	50	43	♂	5.8	5.8	100	m
	51	18	♀	8.4	7.5	89	Ⅰ
	52	29	♂	7.6	7.3	96	
	53	29	♀	7.1	7.7	108	
deficient lacrimation	54	19	♀	5.3	5.5	87	n
	55	23	♂	5.7	5.7	100	Ⅰ
	56	30	♂	9.9	7.4	75	Ⅰ
	57	28	♀	8.2	7.4	90	m
	58	30	♂	16.6	9.2	55	Ⅱ
	59	30	♂	5.8	4.7	81	Ⅰ
	60	25	♂	10.6	10.9	103	

1. Cases with normal lacrimation

In 21 cases, total riboflavin concentration in blood was 6.27±1.17γ% and the bound form 5.08±1.11γ%. Ester ratio was 78.1%. These are lower than the normal, and much lower than the 30 cases of k.s.d.. Existence of certain cases in which ester ratio is remarkably low shows that there are some obstruction in riboflavin metabolism, since the bound form is the active one of riboflavin. In 10 cases out of the above 21, a certain symptom of ariboflavinosis was mentioned. The curative effect was tested for 15 cases by administering riboflavin. Riboflavin contents in blood of them at the first examination are given in Fig. 3.

There were 12 cases which reached a complete healing (●), while 3 cases (×) were

keratitis r	keratitis l	pericorneal congestion	corneal vascularization	glossitis	cheilosis	angular stomatitis	dermatitis	treatment with riboflavin
###	+	−	−	###	###	+	###	### (effective)
+	##	+	+	−	+	−	−	###
+	+	−	−	−	−	−	−	###
+	##	+	+	##	−	−	−	###
+		+	±	−	−	−	−	###
##	##	+	###	−	−	−	−	###
##	##	+	###	−	−	−	−	###
+	+	+	+	###	###	###	+	###
###	##	##	##	##	−	−	−	+
##	##	−	−	+	###	##	−	###
+	+	−	−	−	−	−	−	###
###	###	−	+	+	+	+	###	###
###	###	−	###	±	−	−	−	
−	##	+	−	−	+	−	+	
##	##	+	−	+	##	−	+	
##	##	−	−	−	−	−	−	
+	+	+	−	−	−	−	−	−
+	+	−	−	−	##	−	−	
###	###	+	+	−	−	−	−	
##	##	##	##	###	+	+		
+	+	+	−	−	−	−	−	−
+	+	+	−	−	−	−	−	−
##	##	+	−					+
##	###	+	+	−	−	−	−	−
+	+	+	+	−	−	+	−	−
###	###	+	+	+	+	+	−	−
###	###	+	+	−	−	±	−	
##	###	##	##	−	+	+	−	+
##	##	+	−	−	−	−	−	

in vain by treatment with riboflavin. The riboflavin concentrations in blood of successful 12 cases at the first examination are: -

total 5.58±1.01γ%, bound form 3.97±1.38γ%, ester ratio 72% ; these are all very low as compared with the normal value.

From these facts, it is concluded that there exists, in riboflavin metabolism, a certain obstruction, which will be verified by loading test. Moreover, in all 12 patients k.s.d. was cured by the supply of riboflavin and riboflavin metabolism was recovered at the same time.

Case No. 31 14 age. female. A severe ariboflavinosis was seen in various region (cheilosis, glossitis etc.). At the first examination, the total riboflavin in blood was as low

Fig. 2 The riboflavin concentration in blood. All 30 cases

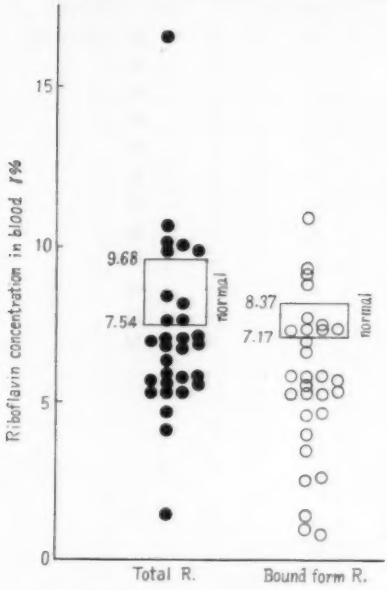
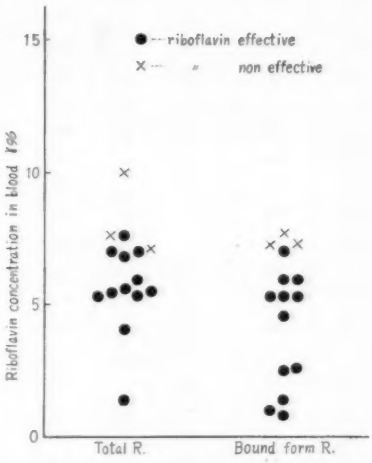
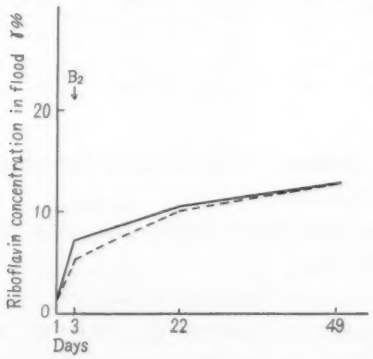


Fig. 3 The riboflavin concentration in blood in cases with normal lacrimation.



as 1.4%. After 10 days continual injection of free riboflavin (10 mg.), the disease in the corneal region was completely cured. Another 10 days injection of 20 mg. FMN made her fully relieve of the various symptoms of ariboflavinosis. Fluctuation of riboflavin concentration in blood is shown in Fig. 4.

Fig 4. Fluctuation of riboflavin concentration in blood. Case No. 31



Case No. 33 25 age, female, The patient showed a slight abnormal Ist. type riboflavin metabolism at the first examination, but she was completely recovered by 10 days continual injection of FMN 20 mg., with the recovery of loading test. (Fig. 5)

Fig. 5 Case No. 33 Fluctuation of riboflavin concentration in blood.

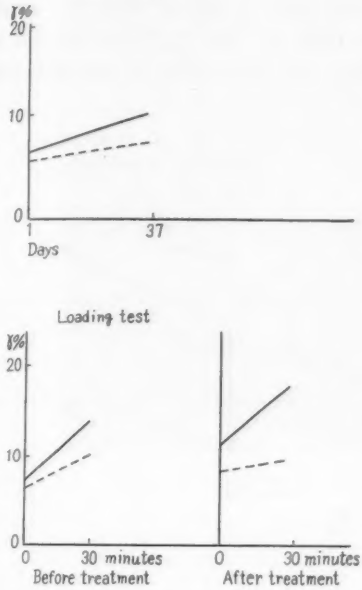
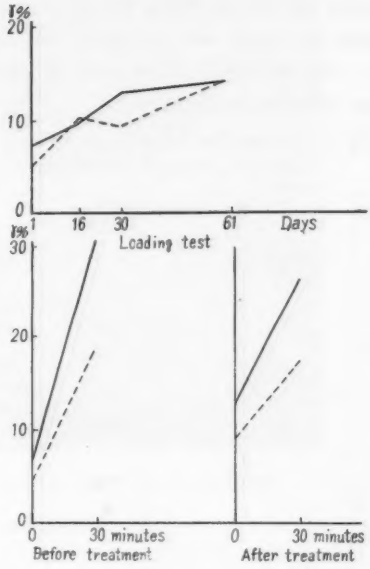


Fig. 6 Case No. 35 Fluctuation of riboflavin concentration in blood.



Case No. 35 24 age, female. The patient showed a mixed abnormal type of riboflavin metabolism, and ester-ratio was as low as 66%. This means an obstruction in utilization of riboflavin. The corneal region and riboflavin metabolism were recovered by 20 days injections of FMN 10mg.. (Fig. 6)

There were three cases in which the patients had the normal lacrimation, but the treatment with riboflavin had no effect. In these cases, the disease may probably be occured by exhaustion such as over-work or coldness, 2 cases of them were suffering from tuberculosis. The important symptom of this case was the severe injuries in the epithelium in the lower part of the cornea. Instillation of cortisone and rest in body proved a good curative effect and brought a complete recovery. These cases have, presumably, something to do with tuberculosis.

2. Cases of deficient lacrimation

Total riboflavin concentration in blood was 5.7%-16.6% (average $9.01 \pm 3.59\gamma\%$). The value are in the normal range. Among them, 5 cases were observed their curative effect as shown in Fig. 7; 2 cases being effective, and 3 non-effective. Even in 2 successful cases, adrenocortical hormone (Interenin) or hypophysen hormone (Praehormone) etc. must be used concurrently, and their recovery took longer time than in the group which had the normal lacirmation. The case No. 55 is the example.

Case No. 55 23 age, male. At the first examination, epithelial deficits were observed in the bulbar conjunctiva and in the corneal region corresponding to the palpebral fissure.

The patient was feeling dryness of the eyes, itching and photophobia. The discharge was very stringy. The riboflavin concentration in blood was as low as 5.7γ%. After 9 days injection of 20 mg. FMN, the blood concentration was raised to 9.4γ%. (Fig. 8) Another Vitamin B_2 mixed with Interenin was injected. The right eye was cured but the left was not. The Schirmer's test was 13 mm. for the right eye, but 0 mm. for the left, which means deficient lacrimation.

Fig. 7 The curative effect of riboflavin. Case with deficient lacrimation.

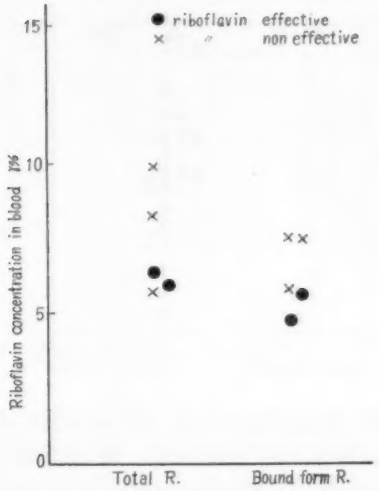
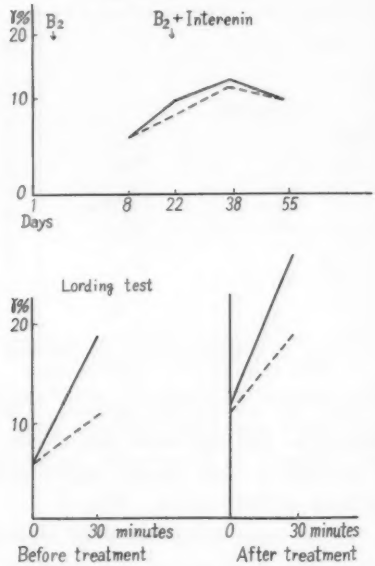


Fig. 8 Case No. 55 Fluctuation of riboflavin concentration in blood.



As a local treatment to the left eye, lacrimal canaliculi was cauterized by a diathermy needle, and by these operation, the patient was completely cured. At that time, a part of lacrimal gland was taken out to make histological examination. The lacrimal gland showed a degeneration of the glandular parenchyma (atrophy and vacuolen degeneration). (Fig. 9)

This is a most conspicuous example in which riboflavin was effective to the eye with normal lacrimation, but was non-effective to the eye with abnormal lacrimation.

Case No. 56 30 age, male. Patient had had foggy vision and has been feeling dyness of the eyes and in the month for one month. K.s.d. was found on both eyes. Riboflavin concentration in blood was normal value, but the loading test showed Ist type abnormality. After 10 days injections of FMN 10 mg. mixed with Interenin, the loading test was recovered to normal but k.s.d. was unchanged. (Fig. 10) Schirmer's test was 0 mm.. After 10 days injections mixed with Praehormone and Methionin (daily 2 g.) region on the corneal epithelium was slightly recovered but had not been cured completely.

Fig. 9 Lacrimal gland in Case No. 55:showing a degeneration of the glandular parenchyma.

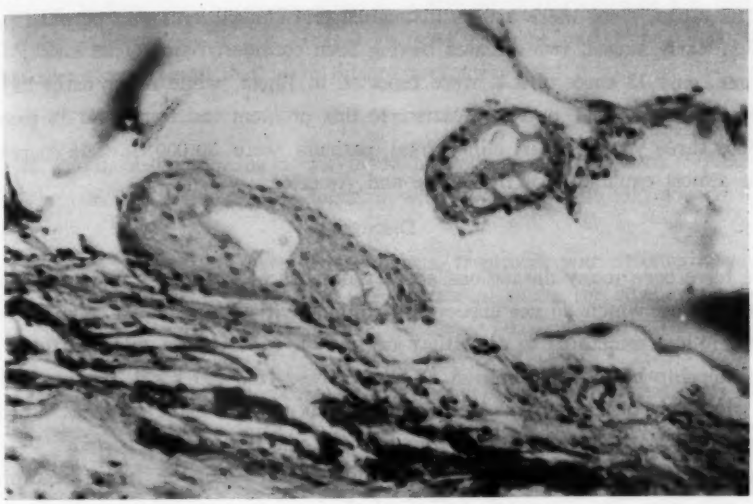
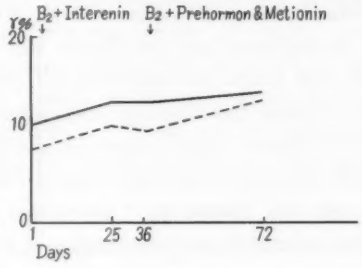
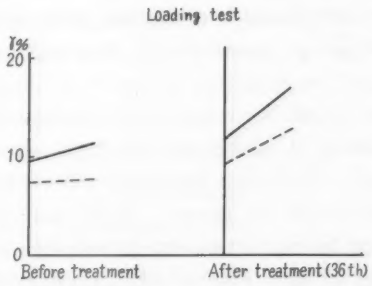


Fig. 10 Case No. 56 Fluctuation of riboflavin concentration in blood.





Clinical symptom and courses of Case No. 57 and 58 were similar as Case No. 56.

In the cases which have deficient lacrimation (as shown Case No. 55, 56, 57, 58) course and symptom was different in the case of normal lacrimation. It may be considered as kerato-conjunctivitis sicca (k.s.).

K.s. shows deficient lacrimation and pitting or gray foci in the epithelial layer of the cornea. In Japan, since there are no distinguishable clinical symptom between k.s.d. and k.s. in their early stages, two diseases having been considered simply as k.s.d.. Up to the present time, only 13 cases of k.s. were reported in Japan, while many cases in Europe.

The author has paid much attention to this problem and found out 16 cases of k.s. during these three years in our clinic (total patients were 30,000). The percentage of patients is almost equal to that in Europe and America.

Discussion

There have been many discussions about the cause of k.s.d. in Japan, especially about the idiopathic cause which do not ensue with conjunctivitis or trachoma. Some investigators argued that it had something to do with "kakke" or tuberculosis, and the others discussed on the relationship between Vitamin A or idiosyncrasy etc. and k.s.d.. Among them, the theory of riboflavin deficiency was dominant, but has no conclusive evidence. Furthermore, many exceptional cases in which riboflavin was non-effective were reported.

As stated above, in cases which had a normal lacrimation, the blood concentration of riboflavin was very low (average $6.27\pm1.17\gamma\%$) and the bound form was much lower (average $5.08\pm1.11\gamma\%$), and the ester ratio was 78.1%. These, together with the result of the loading test, indicate an abnormal riboflavin metabolism. Normal metabolism was recovered. in this case, after the supply of riboflavin, and the disease was cured completely.

These results may indicate that almost all cases of k.s.d. which had shown a normal lacrimation will be aroused by an abnormal riboflavin metabolism.

On the other hand, a kind of disease which causes an abnormal change in the corneal epithelium and is connected with nutrition were often reported in Asian countries.

Nanagas (Phillipine) reported the disease as "nutritional dystrophy of the corneal epithelium", Muthayya (India) named it as "maceration of the corneal epithelium" and Metiva reported it as "another type of superficial changes in the essential corneal epithelium as an entity of ariboflavinosis". (Symptoms were somewhat different.) These workers stated that the disease is caused by the deficiency of Vitamin B or Vitamin B_2 complex.

The symptom of k.s.d. in Japan is similar to that of these diseases in Asian countries. Moreover, deficiency of Vitamin B_2 causes not only k.s.d. which has normal lacrimation, but all these corneal dystrophy. Therefore, the author believes that the diseases in Nanagas, Mathayya are identical with k.s.d. in Japan. While the cases which were diagnosed clinically as k.s.d. with deficient lacrimation showed degeneration in lacrimal gland, accordingly, they may be considered as an early stage of keratoconjunctivitis sicca.

Because of absence of general changes and filaments of the corneal surface in the Ist. and 2nd. type in the classification of Sjøgren's Syndrome made by Gifford, it is very difficult to distinguish k.s. and k.s.d.. Moreover, ariboflavinosis added in k.s. makes it's clinical symptoms more complicated.

Many cases of keratoconjunctivitis sicca were reported in abroad, but only 13 cases were in Japan, and as a rare disease, since it is difficult to discriminate k.s. and k.s.d. in it's early stage. Frequency of k.s. clinical visitants (Eguchi and Funazu), one case in every 3,000 was almost equal to that in Europe and America.

Conclusion

1. In all cases, obstruction in riboflavin metabolism was certified, on the basis of the low riboflavin concentration in blood together with low ester ratio and abnormal result of loading test.

2. In 15 cases out of 21 of normal lacrimation, treatment with riboflavin was effective in 12 cases and non-effective in 3 cases. In successful cases, the riboflavin concentration at the first examination showed a remarkable decrease, i.e. $5.58\pm1.01\gamma\%$ in total, the bound form $3.97\pm1.38\gamma\%$ and ester ratio 72%.

The obstruction of riboflavin metabolism was enhanced in loading test. By the supply of riboflavin, the concentration in blood increased and the normal result was obtained in loading test, then the disease was cured.

It is evident, from the results of these 12 cases of k.s.d., that the disease is caused by the obstruction in riboflavin metabolism. However, in 3 cases in which riboflavin was non-effective, the cause was not certified but probably due to tuberculosis.

3. In some cases of deficient lacrimation (7 cases), total riboflavin concentration in blood is $9.01\pm3.59\gamma\%$ in average, bound form of it is $7.17\pm2.34\gamma\%$ in average. The values are in a normal range. In 2 cases of deficient lacrimation, riboflavin was slightly effective but in 3 cases was noneffective. These are considered as to be in an early stage of keratoconjunctivitis sicca for the reason of deficient lacrimation and clinical signs.

Summary

Riboflavin metabolism has been investigated in 30 cases of keratitis superficialis diffusa.

The group of patients who have normal lacrimation shows an abnormal riboflavin metabolism and is recovered by the supply of riboflavin. It is caused by ariboflavinosis.

In another group of patients who have deficient lacrimation riboflavin metabolism is usually normal and the supply of riboflavin has no effect. Therefore, it may be an early stage of keratoconjunctivitis sicca.

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Correction

In the article "Incorporation of inorganic S^{35}-sulfate into sulfated mucopolysaccharides in the rabbit cornea" by Dr. M. Suda in the 2nd issue, 1958, the photograph of Fig. 2 (page 174) was accidentally inverted.

Editorial News

The sixty second Japan Ophthalmological Congress was held at the Public Hall in Niigata City, on May 16, 17 and 18, 1958. The Chairman was Prof, Masakichi Mikuni M.D. of Niigata University. Besides ninety ordinary speaches one special and a symposium were presented. Guest speakers of foreign conuntries were Prof. A.B. Reese of Colombia University and Prof. R. Thiel of Frankfurt a.M.

Special speach:

Some problems in the diseases of fundus
Prof. Yoshiharu Yoshida, Nagoya City University

Symposium: Pathogeny and treatment of squint
Prof. Tsunekazu Yuge, Kyoto Pref. University
Dr. Makoto Hiroishi, Kyushu University
Dr. Junichi Nakagawa, Sapporo City Hospital

Guest Speaches:

1. Tumour of the eye
   Prof. A.B. Reese, Colombia University
2. Ocular manifestations in diabetes mellitus
   Prof. R. Thiel, Frankfurt a.M.

Ordinary speaches:

1. Slow potential of the retinal cones
   Dr. Yuichiro Sugita, Nagoya Univ.
2. Effect of ACTH on ERG of the rabbit
   Dr. Toshio Yamane, Kyoto Univ
3. Effect of CS_2 on the cone action potential
   Dr. Yoshibumi Seto, Kansai Med. College
4. ERG of the eye of anomalous color sense accompanied by the damage of the optic nerve
   Dr. Takashi Suzuki et al., Iwate Med. College
5. Effect of amino-acid on the tissue respiration of the retina
   Dr. Koku Kozaki, Osaka Med. College
6. Effect of amino-acid and its amine on regeneration of the visual purple
   Dr. Yoneko Muraji, Osaka Med. College
7. Distribution of phosphatase in the retina
   Dr. Shizuo Akiya, Kanazawa Univ.
8. Histological studies on the pigment epithelium and the Müller's fiber in the retina
   Dr. Seiwa Iga, Osaka Univ.

9. Electron microscopic studies on the fine structure of the pigment epithelium and choriocapillary layer
Dr. Shinobu Iwaki, Kurume Univ.
10. Electron microscopic studies on the retinal artery
Dr. Takeo Maeda, Kurume Univ.
11. Clinical measurement of caliber of the blood vessels in the normal eyes
Dr. Midoriko Tsunematsu, Jikei Med. College
12. Effect of streptohydrazide on caliber of the retinal blood vessels
Dr. Ken-ichi Nagase et al., Niigata Univ.
13. Effect of fever on caliber of the retinal vessels
Dr. Hajime Tsuchiya, Niigata Univ.
14. Studies on the subretinal fluid
Dr. Atsumi Nakano, Hiroshima Univ.
15. Studies on capillary fragility in the ocular diseases
Dr. Yoshiko Kojima, Nagoya City Univ.
16. Biomicroscopic changes of the posterior conjunctival arteries in hypertension
Dr. Kenji Takahashi et al, Nihon Univ.
17. Studies on the blood vessels of the bulbar conjunctiva in hypertension
Dr. Shizuo Sato, Keio Univ.
18. Measurement of caliber of the retinal vessels and retinal blood pressure in essential hypertension
Dr. Takashi Nakashizu, Niigata Univ.
19. Relationship between the retinal blood vessels and heart changes in the old
Dr. Shogo Hiwatari et al., Doai Memorial Hospital, Tokyo
20. Studies on pupillary reflex by inframed photography
Dr. Torajiro Hiraoka, Nippon Med. College
21. Clinical studies on serum protein, liver function and the changes of cerebrospinal liquor in idiopathic uveitis and Behçet's disease
Dr. Shozo Majima, Chiba Univ.
22. Experimental studies on mucocutaneous ocular syndrome
Dr. Tadashi Kato, Univ. of Tokyo
23. Electron microscopic studies on sympathetic ophthalmia (III)
Dr. Hiroshi Ikui et al., Kurume Univ.
24. Studies on the normal human conjunctiva as revealed in ultrasection by electron microscopy
Dr. Yoshiko Shibuya, Hokkaido Univ.
25. Studies on follicles in the conjunctiva: (V)
Dr. Norio Yanagaki, Nihon Univ.
26. Electron microscopic studies on trachomatous follicles
Dr. Yoshiko Miura, Sapporo Univ.
27. Trachoma virus in section revealed by electron microscopy (III)
Dr. Yukihiko Mitsui et al., Kumamoto Univ.
28. Serial passage of trachoma virus on chorioallantoic membrane of fertile chick egg
Dr. Shuyu Otsuka, Hokkaido Univ.
29. Relationship between adenovirus and EKG
Dr. Chie Tanaka & Hiro Watanabe, Tokyo Women's Med. College
30. Studies on the occurrence of punctate opacities in the cornea: inoculation-test with adenovirus
Dr. Kaichi Wakui, Univ. of Tokyo
31. Bacterial flora in the cul-de-sac
Dr. Teruyo Yamazaki, Gifu Pref. Univ.
32. Symbiosis of corynebacterium xerosis and staphylococci in the cul-de-sac
Dr. Hiroshi Shimizu, Okayama Univ.

33. Radiotherapy of pterygium using β-ray
Dr. Yoshisuke Saito, Yokohama Univ.
34. Electron microscopy of the corneal tissue
Dr. Taeko Ishida, Toho Univ.
35. Effect of stress on the mitotic activity of the corneal epithelium
Dr. Kunio Tanaka, Osaka City Univ.
36. Corneal metabolism in hetero-kerato-plasty
Dr. Kyoichiro Nonaka, Tokyo Med. College
37. Corneal immunity in vaccinia keratitis
Dr. Ichini Okamoto et al., Yamaguchi Med. College
38. Mucopolysaccharides in the cornea and sclera
Dr. Ko Takaku, Tohoku Univ.
39. Studies on experimental incipient cataract
Dr. Tsugihiko Tokunaga, Nagasaki Univ.
40. Studies on the genesis of naphthalene cataract
Dr. Takashi Mizukawa et al., Tokushima Univ.
41. Studies of diabetic cataract
Dr. Fumio Kandori et al., Tottori Univ.
42. Effect of low oxygen partial pressure on the lens
Dr. Masayuki Hoshina, Tokushima Univ.
43. Studies on the isolation and refining of lens proteins
Dr. Yasuo Minura, Osaka Univ.
44. Studies on lens glycogen
Dr. Toshiro Kamei, Gumma Univ.
45. Tonography
Dr. Yasusaburo Hata, Keio Univ.
46. Studies on the pressure tonography in glaucoma
Dr. Hiroshi Tabara, Kyushu Univ.
47. Relationship between adrenocortical hormone and ocular tension
Dr. Hiroshi Kozaki, Osaka Univ.
48. Relationship between water content in blood and drinking-test
Dr. Seikyo Yonebayashi, Kyoto Pref. Univ.
49. Penetration of Diamox into the ocular tissues and changes of ocular tension
Dr. Sho Kusube, Osaka City Univ.
50. Effect of anthranilic-acid on ocular tension
Dr. Shozaburo Yasuda, Wakayama Med. College
51. Influence of Pacatal to ocular tension
Dr. Ryukichi Morinobu, Okayama Univ.
52. Influences of the sympathetic nerve to autonomic adjustment function of occular tension
Dr. Takeshi Suzuki, Okayama Univ.
53. Prognosis of simple glaucoma
Dr. Keiu Suda et al., Kumamoto Univ.
54. Clinical studies on the injection of the optic disk
Dr. Yozo Suzuki, Jikei Med. College
55. Experimental studies on brain allergy in relation to the genesis of retrobulbar neuritis
Dr. Shuji Sugawara, Tohoku Univ.
56. Experimental studies on ocular allergy (III)
Dr. Shogo Kojima, Gifu Pref. Med. College
57. Changes of reduced materials of B.T. in the retina, ciliary body and aqueous humor by loading of cortisone
Dr. Zenichi Kubota, Nagoya Univ.
58. Influences of pilocarpin and atropin on the concentration of Na, K in aqueous humor
Dr. Teruko Yoshizawa, Tokyo Med. and Dent. Univ.

59. Phosphate metabolism in aqueous humor
Dr. Hiroshi Senoo, Iwate Med. College
60. Experimental studies on phosphate metabolism in the optic nerve of dog using P^{32}
Dr. Akio Kobayashi, Kobe Med. College
61. Experimental studies on the changes of the optic nerve in vitamin A deficiency
Dr. Yuzo Kikuchi, Chiba Univ.
62. Histochemical studies of VA & VB_2 in the retina
Dr. Seiichi Yamada et al., Hirosaki Univ.
63. Influence of the sympathetic denervation on the VB_1 distribution in the eye
Dr. Yasuro Goto, Amagasaki Hospital
64. Effect of adrenocortical hormone on distribution of VB_1 in the eye
Dr. Eiichi Miyazaki, Kyoto Univ.
65. Penetration of P^{32}-laveled FMN & FAD into the ocular tissues
Dr. Toshiyuki Hishiki, Nagoya Vnlv.
66. Effect of VB_2 on the corneal metabolism (I)
Dr. Hidehiro Funazu, Univ. of Tokyo
67. Ocular changes in VB_{12} deficiency
Dr. Hiroshi Takahashi, Hirosaki Univ.
68. Studies on the pulse-wave of ocular pressure using ultrasonics
Dr. Isaku Suzuki et al., Osaka Pref. Hospital
69. Experimental studies on the effect of ultrasonics on the biological functions of the ocular tissues
Dr. Katsu Okuda, Mie Univ.
70. Action potential of the nucleus of the oculomotor nerve
Dr. Masashi Shimooku, Kobe Med. College
71. Effect of Adaptinol on experimental pigment degeneration of the retina
Dr. Kazuo Matsushita, Osaka
72. Fundus changes caused by experimental aortic stenosis
Dr. Kinnosuke Hirose et al., Nagasaki Univ.
73. Embryological studies on the lacrimal apparatus of fetus
Dr. Takayuki Senoo, Nippon Med. College
74. Experimental studies on the diagnosis of orbital tumours
Dr. Torao Tanoue, Kagoshima Univ.
75. Studies on the aqueous lens
Dr. Reiji Suzuki, Shinshu Univ.
76. Objective measurement of ocular-axis of living bodies
Dr. Kiyonori Amano, Juntendo Univ.
77. Studies on myopia by the measurement of ocular-axis using X-ray photography
Dr. Fuku Sugata, Tokyo Med. & Dent, Univ.
78. Studies on night myopia (I)
Dr. Hiroshi Murata, Fukushima Med. College
79. Studies on the insufficiency of the ciliary muscle noteworthy in the hygiene of close-sighted work
Dr. Ayao Koyama et al., Hiroshima
80. A new sensitivitometer of visual field and its application
Dr. Jun Sugawara, Takamatsu
81. Studies on the objective measurement of visual acuity (IX)
Dr. Ryoichi Yamaji et al., Osaka Med. College
82. Studies on idiosyncratic and cooperative natures of differential stimulations in visual perception (II)
Dr. Sumiyoshi Akutsu, Nara Med. College
83. Effect of color light on visual functions (III)
Dr. Noboru Ando, Nagoya Univ.
84. Mental fartigue and visual function
Dr. Keishin Mizutani, Nagoy Univ.
85. On the electrical sensitivity of the human eye for constant current pulses of varying

frequencies (IV)

Dr. Keihu Murakami, Kanazawa Uciv.

86. Studies on color sensation by ERG

Dr. Hitohiro Sakanashi, Tokyo Med. College

87. Studies on the changes of melanophore expanding and contracting hormone by adaptation

Dr. Fun Yoshida, Gumma Univ.

88. Space angle test

Dr. Hiroshi Kawaoka, Kyushu Univ.

89. Treatment of amblyopy in squint

Dr. Koichi Adachi & Shota Inatomi, Kyoto Pref. Univ.

90. Studies on binocular movement of squinting eye

Dr. Kazuo Kato, Juntendo Univ.

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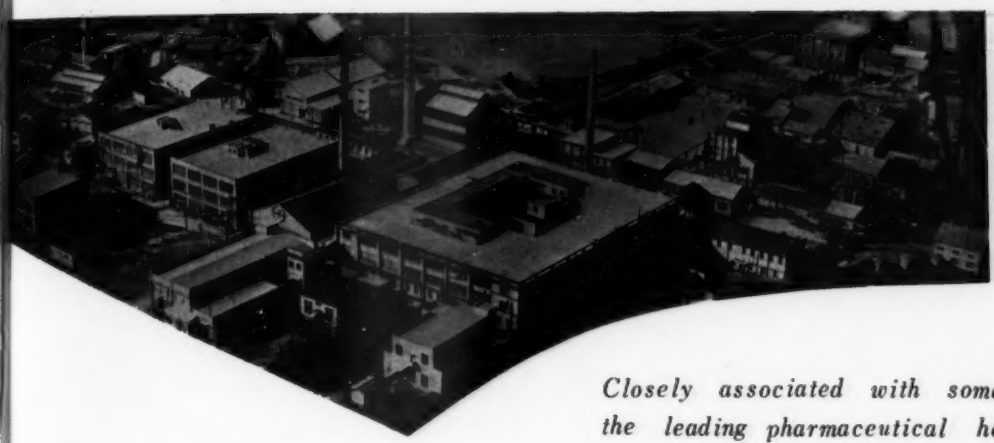

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